American

POTATO JOURNAL

Volume 35 *July 1958* *Number 7*

CONTENTS

NEWS AND REVIEWS

Official Publication of

THE POTATO ASSOCIATION OF AMERICA

NEW BRUNSWICK, NEW JERSEY, U. S. A

American Potato Journal

PUBLISHED BY
THE POTATO ASSOCIATION OF AMERICA
NEW BRUNSWICK, N. J.

Price $4.00 per year in North America; $5.00 in other countries.
Not responsible for free replacement of non-delivered or damaged issues after 90 days.

Entered as second class matter at New Brunswick, N. J., March 14, 1942 under Act of March 3, 1879. Accepted for mailing at special rate of postage provided for in section 412. Act of February 28, 1925, authorized on March 14, 1928.

FOLIAR APPLICATION OF UREA TO POTATOES[1]

E. Francis Button[2] and Arthur Hawkins[3]

There has been considerable interest in recent years in foliar application of urea as a means of supplying part of the nitrogen for potatoes. Studies in several states (2)[4], (4), (5), (7) have demonstrated the response of potatoes to foliar applications of urea; but only in the Maine (7) and Rhode Island (6) experiments were comparisons made of applying the same amount of additional nitrogen by other methods.

The purpose of the investigation reported here was to determine the effect of foliar applications of urea as a means of supplying part of the nitrogen for potatoes under Connecticut conditions on the quantity and quality of the tubers produced and on the nitrogen content of the leaflets.

Since it is customary to spray potatoes weekly for insect and disease control, and since urea can be included in the spray mixture, the only cost is for the material. This study was designed to compare the efficiency of supplying part of the nitrogen as urea in weekly foliar applications as compared with other methods of supplying part of the nitrogen.

Materials and Methods

Experiments were conducted on two commercial potato farms in the Connecticut River Valley in both 1954 and 1955 on fields which had a history of continuous cultivation for many years and which had been in potatoes the previous two years.

The soil on Farm A is classified as Enfield very fine sandy loam and on Farm B as Enfield silt loam. The analyses of the soil from both locations, as determined by a modified Morgan Method (3), were comparable and typical of potato soils in the Connecticut River Valley. Soil from both locations, obtained prior to lime applications in May 1954, were pH 4.8 to 4.9; the readily available nutrients were medium to medium high in nitrate nitrogen; very low in calcium; medium in magnesium; high in phosphorus; and medium in potassium. Samples obtained in May 1955 ranged from pH 5.0 to 5.2 and were very low in nitrate nitrogen.

Katahdin potatoes were planted in plots 4 or 6 rows wide, with 2 or 4 rows for harvest, and 28 or 44 feet long in experiments 1 and 2 respectively, in 1954, and 40 feet long in 1955. The plots were replicated six times in a Latin Square design. Two feet at the ends of each plot were discarded at harvest time.

All plots received a uniform side-band application at planting time of 180 to 200 pounds per acre each of P_2O_5 and K_2O, and 30 pounds per acre of water soluble MgO. The source of potash was subplotted, one-half of the rows received KCl and the other rows K_2SO_4. The source of potash had no significant interaction effect on the nitrogen treatments.

[1]Accepted for publication Oct. 30, 1957.
Contribution from Agronomy Section of Storrs Agricultural Experiment Station, Storrs, Conn.

[2]Formerly Research Assistant in Agronomy, University of Conn.; presently Agronomist, Connecticut State Highway Dept., Portland, Conn.
[3]Agronomist, Storrs Agricultural Experiment Station, Storrs, Conn.

[4]Numbers in parenthesis refer to the literature cited.

The amount of nitrogen applied in the row at planting time varied with the treatment. In all cases the row application of nitrogen consisted of 20 pounds per acre from sulfate of ammonia, 10 pounds from castor pomace, and the balance from urea. The assisted-feed planter, equipped with an endless belt type of fertilizer attachment, applied quantities of fertilizer mixtures weighed out according to plot lengths, in the row side placement.

Side-dressed treatments were applied by hand in bands about 2 to 3 inches wide on each side of the row, about 4 to 6 inches from the center of the row when the plants were 10 to 12 inches high in 1954, and 8 to 10 inches in 1955. Ammonium nitrate was the nitrogen source side-dressed on Farm A, whereas ammonium nitrate and urea were compared on Farm B in 1954. In 1955, urea was used for the side-dressed treatments on both farms. The broadcast applications of urea in 1955 were made by hand, prior to plowing on Farm B, and after plowing on Farm A.

Foliar Application of Urea

The foliar applications of urea were applied at weekly intervals in 80 gallons of water per acre. In 1954, the first application was made when the plants began to fill the row and were nearly in full bloom. In 1955, the first foliar application for treatment 2 was applied when the potatoes started to fill the row and were in the late bud stage; applications for treatments 3 and 4 were started two and four weeks later, respectively.

A portable sprayer was used, equipped with T-jet conical nozzles, and operated by compressed air at a constant pressure of 35 lbs/sq. in. This apparatus was satisfactory for applying a measured volume of spray material to a given length of row. A long boom was used to eliminate travel within the inner four rows of the 6 row plots.

Leaflet Sampling

The 4th leaf below the growing tip was sampled from 24 representative plants from each replicate of the treatments sampled. In 1954, leaflets were sampled at weekly intervals at both locations with the exception of the last sampling which was delayed for two weeks. Farm A, as shown in table 4, was sampled one week earlier than Farm B. In 1955, leaflets were sampled at two week intervals. Leaflets from the earlier planted locations were sampled one week earlier than from the later planted plots, as shown in table 5. The leaves were washed in 0.1 NHCl, washed in running tap water, and rinsed in distilled water. The leaflets were stripped from the rachises, dried, ground through a 40 mesh screen, and stored in stoppered bottles. Samples were analyzed for total nitrogen (less nitrate nitrogen) (1). The analyses of leaflets from the treatments sampled in 1954 are shown in table 4 and figure 1; and from the 1955 treatments in table 5 and figure 2.

Tuber Quality

Samples of 10 to 14 representative tubers 2½ inches to 3 inches in diameter were obtained from each replicate at harvest time. Specific gravity determinations made by the salt solution method are given in the tables of yields. Tubers were examined for hollow heart.

TABLE 1.—*Effect of applying additional nitrogen by foliar application and and by side-dressing on Katahdin potato yields—Experiment I—Farm A, 1954.*

Nitrogen Applied[1]—Lbs. Per Acre				Yield	Specific Gravity of Tubers
In the Row[2]	Side-dressed[3]	Foliar Spray[4]	Total	Bus./Acre	
1. 60	0	0	60	423	1.076
2. 60	30	0	90	495**	1.077
3. 60	0	30 (6x5 Lbs. N)	90	465**	1.077
4. 60	60	0	120	525**	1.077
5. 60	0	54[5] (9x6 Lbs. N)	114	451*	1.077
6. 60	0	60 (8x7.5 Lbs. N)	120	472**	1.073
L.S.D. .05*				25	
.01**				34	
C.V. = 4.39 per cent					
Side-plots 0	0			259	

[1]All plots received 200 pounds each of P_2O_5 and K_2O and 30 pounds water soluble MgO per acre rate in row side-band placement at planting time. Planted May 13.
[2]Source of Nitrogen in the Row: 10 pounds per acre from castor pomace, 20 pounds from sulfate of ammonia, 30 pounds from urea.
[3]Side-dressed Nitrogen: Ammonia nitrate applied 6/15; plants were 10-12″ high.
[4]Foliar Applications of Urea: Weekly applications of 5, 6 and 7.5 pounds of nitrogen per acre from urea for treatments 3, 5 and 6, respectively. First application on 7/8.
[5]Only nine of the 10 planned foliar applications were made due to decline of the vines; last application on 9/2.

In 1954, these samples were used to determine the effects of various treatments upon after-cooking darkening. A longitudinally-cut quarter from each of 8 tubers from each sample was thinly-peeled, dropped into boiling water in aluminum pans, boiled 20 minutes, removed from the pans, and then placed on white paper and the amount of darkening recorded after the tubers had been exposed to the air for one half hour.

WEATHER AND GROWING CONDITIONS

The temperature for the major portion of the growing season of 1954 was relatively cool and very favorable for potatoes. However, heavy rainfalls brought by the hurricanes of August 31 and September 11 undoubtedly caused considerable loss of available nitrogen by leaching and resulted in the rapid decline and untimely death of the vines at both locations.

The growing season of 1955 was not favorable for potato production. The temperature during June, July, and August was abnormally high, and precipitation was below normal in June and July. Irrigation was inadequate since it was not applied until late July at both locations. Since this period (58 to 78 days after planting) coincides with the critical period of nitrogen absorption of the crop (3) optimum nitrogen up-take

TABLE 2.—*Effect of applying additional nitrogen by foliar applications and by side-dressing as compared to all the nitrogen band-placed at planting on Katahdin potato yields—Experiment 11—1954.*

Farm A

	Nitrogen Applied[1]—Lbs. Per Acre				Yield	Specific Gravity of Tubers
	In the Row[2]	Side-dressed[3]	Foliar Spray[4]	Total	Bus./Acre	
1.	90	0	0	90	484	1.075
2.	90	60	0	150	558**	1.076
3.	90	0	60 (8x7.5 Lbs. N)	150	511	1.076
4.	60	90	0	150	521	1.075
5.	60	0	60[5] (8x7.5 Lbs. N)	120[5]	493	1.074
6.	150	0	0	150	543	1.076
L.S.D.		.05*			39	
		.01**			54	
C.V. = 6.3 per cent						

Farm B

	In the Row[2]	Side-dressed[3]	Foliar Spray[4]	Total	Early Harvest	Final Harvest	Early Harvest	Final Harvest
1.	90	0	0	90	586	593	1.075	1.076
2.	90	60	0	150	645**	674**	1.076	1.076
3.	90	0	60 (8x7.5 Lbs. N)	150	639*	655**	1.075	1.076
4.	60	90	0	150	647**	651*	1.077	1.077
5.	60	0	9x7.5[5]	127.5[5]	604	577	1.075	1.074
6.	150	0	0	150	668**	667**	1.077	1.076
L.S.D.		.05*			40	44		
		.01**			54	60		
C.V.					5.26%	5.72%		

[1]See footnote 1, table 1. Farm A planted May 14; farm B planted May 20.
[2]Source of Nitrogen in the Row: 10 pounds per acre from castor pomace, 20 pounds from sulfate of ammonia, balance from urea.
[3]Side-dressed Nitrogen: Ammonia nitrate applied on Farm A on June 15 when plants were 10-12" high; ammonia nitrate and urea on split plots on Farm B, June 21.
[4]Weekly applications of 7.5 pounds N per acre from urea, first applications made on 7/9 on Farm A; 7/12 on Farm B.
[5]Due to the early decline of vines, only 8 of 12 planned applications for Treatment 5 were made on Farm A, last application on 8/27; only 9 of 12 were made on Farm B, last on 9/2.

was not possible until the substantial precipitation received during the first 10 days of August encouraged rapid, vigorous vine growth. Hurricane rains of August 11, 12, 13 totalling 5.02 inches, and a storm on the 18th during which 4.77 inches were recorded before the rain gauge was damaged, resulted in the very rapid decline and early death of the vines at both locations.

TABLE 3.—*Effect of applying varying amounts of additional nitrogen by foliar application as compared to additional nitrogen side-dressed or broadcast on potato yields—1955.*

Farm A

Nitrogen Applied[1]—Lbs. Per Acre				First Weekly Foliar Application Date	Yield	Specific Gravity of Tubers
In the Row[2]	Side-dressed	Foliar Spray[4]	Total		Bus./Acre	
1. 90	0	0	90		335	1.058
2. 90	0	60 (8x7.5 Lbs. N)	150	7/8	331	1.058
3. 105	0	45 (6x7.5 Lbs. N)	150	7/21	352	1.059
4. 120	0	30 (4x7.5 Lbs. N)	150	8/3	354	1.058
5. 120	30 S.D.[3]	0	150		336	1.059
6. 90	60 B.C.[5]	0	150		357	1.060
L.S.D. C.V. = 7 per cent					N.S.	

Farm B

In the Row[2]	Side-dressed	Foliar Spray[4]	Total	Date	Yield				
					Early Harvest	Final Harvest	Difference	Early Harvest	Final Harvest
1. 90	0	0	90		421	444	23	1.057	1.058
2. 90	0	60 (8x7.5 Lbs. N)	150	7/14	351	419	68	1.055	1.054
3. 105	0	45 (6x7.5 Lbs. N)	150	7/29	376	462	86	1.053	1.056
4. 120	0	30 (4x7.5 Lbs. N)	150	8/10	397	455	58	1.055	1.059
5. 120	30 S.D.[3]	0	150		399	461	62	1.056	1.058
6. 90	60 B.C.[5]	0	150		401	445	44	1.056	1.057
L.S.D.					N.S.	N.S.	Sig. 1%		
C.V.					9.7%	8.4%			
Sideplots (Adjoining test, same field)									
0	0	0	0			276			

[1]All plots received 180 pounds each of P_2O_5 and K_2O, 30 pounds water soluble MgO per acre, in row side-band placement at planting time. Planted Farm A, May 18; Farm B, May 25.

[2]Source of Nitrogen in the Row: 20 pounds per acre from sulfate of ammonia, 10 pounds from castor pomace, and the balance from urea.

[3]Side-dressed Nitrogen: Urea applied when plants were 8-10" high, June 23, Farm A; June 30, Farm B.

[4]Foliar Application of Urea: Weekly applications of 7.5 pounds of N per acre from urea began for treatment 2 on 7/8 for Farm A, 7/14 on Farm B. Dates of first weekly application for treatment 3 and 4 as indicated.

[5]Broadcast Nitrogen: Applied urea after plowing on May 18 on Farm A; before plowing on Farm B.

TABLE 4.—*The effect of additional nitrogen applied in periodic foliar applications on the nitrogen content of Katahdin potato leaflets in 1954.*

Nitrogen Pounds per Acre			Nitrogen Content of Leaflets Sampled Periodically								
			Farm A[1]					Farm B[1]			
In the Row		Foliar[4]	Date Sampled[2]								
			7/26	8/2	8/9	8/16	8/30	8/2	8/9	8/16	8/30
			Nitrogen[3] (Per cent Oven Dry Basis)								
1.	90	0	6.6	6.0	5.9	5.5	3.9	6.6	6.0	5.5	4.1
3.	90	60	6.8	6.5	6.4	6.3	4.9	7.0	6.8	6.5	5.6
			Number of Weekly Foliar Applications of 7.5 Pounds N per Acre Applied Prior to Date Sampled[4]								
3.	90	60	3	4	5	6	8	4	5	6	8

[1]Farms A and B planted May 14 and 20, respectively.
[2]Leaflets sampled 5 to 6 days after foliar applications were made.
[3]Results are averages of duplicate analyses of leaflet material from the potash subplots, reported on the oven dry basis, total nitrogen except nitrates.
[4]Eight weekly foliar applications of 7.5 pounds of nitrogen per acre from urea.

TABLE 5.—*The effect of varying amounts of foliar applied and side-dressed and broadcast supplemental nitrogen on the nitrogen content of potato leafetls—1955.*

Nitrogen Pounds per Acre			Nitrogen Content of Leaflets Sampled Periodically							
			Farm A[1]				Farm B[1]			
In the Row		Foliar[4] or Other	Date Sampled[2]							
			7/26	8/9	8/23	9/6	8/2	8/16	8/30	9/12
			Nitrogen[3] (Per cent Oven Dry Basis)							
1.	90	0	6.5	5.6	6.3	5.0	5.9	6.2	5.4	4.5
2.	90	60 Foliar-applied[4]	6.7	6.2	6.7	5.6	6.3	6.8	6.0	5.0
3.	105	45 " "	6.7	6.0	6.6	5.5	6.4	6.9	6.2	5.1
4.	120	30 " "	6.5	5.9	7.0	5.7	6.1	6.6	6.2	5.2
5.	120	30 Side-dressed	6.6	5.9	6.6	5.3	6.4	6.8	6.0	4.9
6.	90	60 Broadcast	6.8	6.0	6.3	5.1	6.4	6.7	5.9	4.9
			Number of Weekly Foliar Applications of 7.5 Pounds N per Acre Applied Prior to Date Sampled[4]							
2.	90	60 Foliar-applied[4]	3	5	6	8	3	5	7	8
3.	105	45 " "	1	3	4	6	1	3	5	6
4.	120	30 " "	0	1	2	4	0	1	3	4

[1]Farms A and B planted May 18 and 25, respectively.
[2]Leaflets sampled 5 to 6 days after foliar applications were made.
[3]Results are averages of duplicate analyses of leaflet material from the potash subplots, reported on the oven dry basis, total nitrogen except nitrates.
[4]Weekly foliar applications of 7.5 pounds of nitrogen per acre from urea in 8, 6 and 4 sprays for Treatments 2, 3 and 4, respectively.

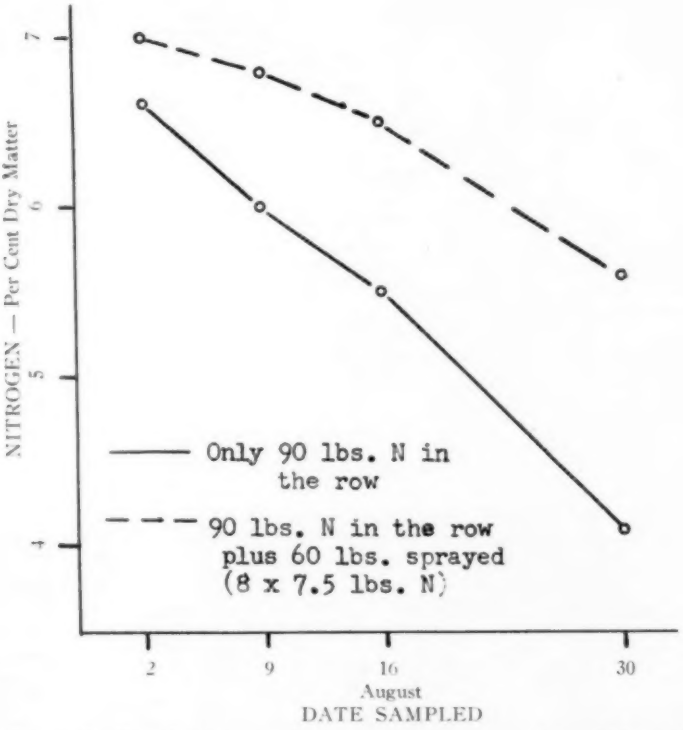


FIGURE 1.—The nitrogen content of potato leaflets from plots which received foliar applied urea (in eight weekly applications of 7.5 lbs. of N applied, 7/12 to 8/27) and from unsprayed plots on four sampling dates, Farm B, 1954. Leaflets sampled 5 to 6 days after foliar applications were made.

RESULTS AND DISCUSSION

EXPERIMENT 1 — 1954

In this experiment, all treatments received 60 pounds of nitrogen per acre in the row at planting time. Comparisons were made of applying either 30 and 60 additional pounds of nitrogen per acre as a side-dressing of ammonium nitrate or as weekly foliar applications of urea, as shown in table 1. With one exception highly significant increases in yield were obtained to additional nitrogen whether applied either as side-dressing or by foliar applications. The yield increases from the side-dressed treatments was significantly greater than the yield increases from the comparable foliar treatment at the 30 pound N per acre level, and highly significantly better at the 60 pound level.

The foliage on plots which received only 60 pounds of nitrogen per acre in the row showed nitrogen deficiency as early as the middle of July, and by August 2 only 6 per cent of the vines in these plots were

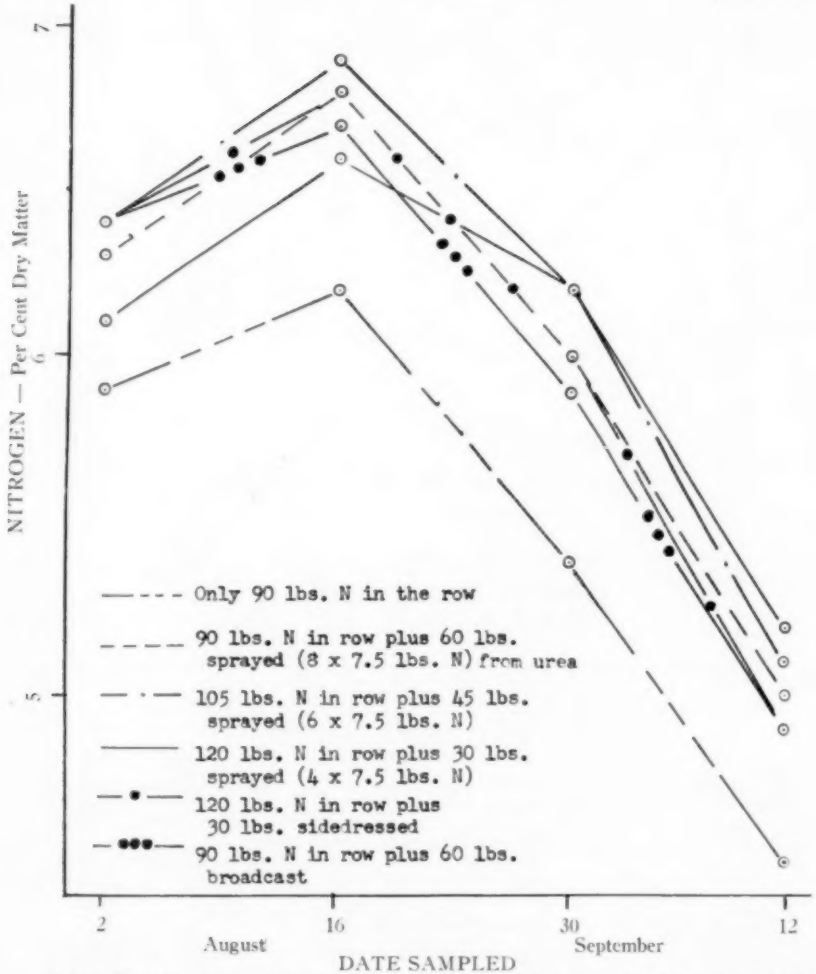


FIGURE 2.—Nitrogen content of potato leaflets sampled periodically from plots which received part of the Nitrogen either in weekly foliar applications of urea at 7.5 pounds of N per acre, or in side-dressed or broadcast applications. Farm B, 1955.

alive. On plots side-dressed with an additional 30 and 60 pounds of nitrogen per acre 42 per cent and 83 per cent of the foliage was alive at this date. The foliage in treatments 3, 5, and 6 which had by August 2 received 20, 24, and 30 pounds of additional nitrogen per acre, through weekly foliar applications, were 29, 47 and 83 per cent alive, respectively.

It is doubtful that much of the later foliar applications of nitrogen were fully utilized because of the early and rapid decline of the vines. The vines in all plots were dead by September 1.

Under the condition of this experiment the delayed application of nitrogen by foliar application on plots which had received only 60 pounds of nitrogen in the row at planting time, was inferior to the side-dressed method.

Additional nitrogen had little or no effect on the specific gravity of the tubers. No hollow hearts were found in the tubers sampled.

Experiment 2 — 1954

In this experiment at two locations in 1954, comparisons were made of the following treatments: (1) applying 60 pounds of nitrogen per acre as a side-dressing or in eight weekly foliar applications of 7.5 pounds of nitrogen in addition to 90 pounds of nitrogen per acre in the row at planting time; (2) applying 90 pounds of nitrogen per acre as a side-dressing or as twelve foliar applications of 7.5 pounds of nitrogen, in addition to 60 pounds of nitrogen in the row; and (3) all the nitrogen, 150 pounds, applied in the row as sideband placement during the planting operation. In addition one treatment received only 90 pounds of nitrogen in the row.

Farm A, 1954

Highly significant increases in yields were obtained to an additional 60 pounds of nitrogen per acre when applied either as a side-dressing or included all in the row at planting time (Table 1). There was a significant yield increase of 47 bushels per acre from the side-dressed treatment as compared with the yield from the treatment which received the additional 60 pounds of nitrogen in eight weekly foliar applications. The yield increase from the latter treatment was only 27 bushels per acre above that obtained from the plots which received only 90 pounds of nitrogen per acre in the row, an increase which was *not* significant.

The yields from treatment 2 — 90 in the row plus 60 side-dressed — was 37 bushels per acre greater than that obtained from treatment 4, 60 in the row plus 90 side-dressed. This difference, however, was not quite significant at the 5 per cent level.

Because of the early decline of the vines, the plots of treatment 5 received only eight of the twelve planned foliar applications in addition to 60 pounds in the row at planting. The yield was about the same as the yields obtained from treatment 1, which received only 90 pounds in the row. Nitrogen deficiency appeared in both treatment 1 and 5 about the middle of August, and by September 9 the foliage was only 4 per cent alive in treatment 1 and 27 per cent alive in treatment 5. The foliage was 76 per cent, 71, 80 and 92 per cent alive in treatments 2, 3, 4, and 6, respectively. Following the hurricane rainfall of over 4 inches on September 11, decline of the remaining live vines of all plots was very rapid.

There was little difference in specific gravity in the tubers from all treatments and no hollow hearts were found.

Farm B, 1954

Highly significant yield increases were obtained from plots which received an additional 60 pounds of nitrogen per acre either (a) as a

side-dressing, (b) in eight weekly foliar applications of urea, or (c) an application of 150 pounds, all in the row at planting time, as compared with plots which received only 90 pounds in the row (Table 2).

Plots which were to receive ninety pounds of nitrogen per acre in twelve foliar applications of urea, in addition to 60 pounds in the row, received only 9 foliar applications and the yield obtained was slightly less than that of the treatment which received only 90 pounds in the row.

The foliage on plots of treatment 1, and on the treatments which received only 60 pounds in the row at planting time plus additional nitrogen, either as a side-dressing or as foliar applications, showed nitrogen deficiency by mid-August. The foliage of plants which received 150 pounds of nitrogen in the row was superior to the foliage in all other plots. The foliage of treatment 1 was nearly all dead by September 13. The vines of treatment 3 and 5 were nearly dead by September 15, and the vines of all the other plots were dead by September 20.

Early *vs.* Late Harvest, 1954

On Farm B in 1954 a study was made to determine the effects of deferred applications of nitrogen by foliar application on tuber development. The vines on half of each plot were pulled on September 9, 1954, but vines not pulled were dead by September 20th as a result of adverse weather conditions. The yield difference between early and late harvests was not statistically significant, however. (Table 2).

Experiment 3 — 1955

The effect of additional nitrogen from urea applied to potatoes, by foliar application, by side-dressing, and by broadcasting, on the yields and quality of the tubers was investigated in 1955 at two locations. Comparisons were made of the following treatments: (1) applying 90 pounds of nitrogen per acre in the row at planting time with an additional 60 pounds in eight foliar applications of urea; (2) applying 105 pounds of nitrogen per acre in the row and an additional 45 pounds as six foliar applications; (3) applying 120 pounds of nitrogen per acre in the row plus 30 pounds as four foliar applications; and (4) applying 120 pounds of nitrogen per acre in the row supplemented with 30 pounds as a side-dressing. A broadcast application of 60 pounds of nitrogen per acre was applied after plowing on Farm A and before plowing on Farm B, in addition to 90 pounds applied in the row in the complete fertilizer. One treatment received only 90 pounds of nitrogen in the row at planting time.

The yield data from Farms A and B in 1955 are summarized in table 3. In 1955 little or no increase in yield was obtained from more than 90 pounds of nitrogen per acre in the row. This was probably a result of the higher than normal temperatures in June, July, and early August, the lack of sufficient moisture early in the season, and the heavy hurricane rains in August of more than 9.79 inches which resulted in considerable loss of available soil nitrogen by leaching, and the early death of the plants.

Farm A, 1955

Nitrogen deficiency symptoms appeared in the foliage of treatments 1 and 2 the first week of August, and yellowing of lower leaves and loss

of vigor occurred in *all* plots after the heavy rains of mid-August. By September 12, 1955, at least 50 per cent of the vines in all plots were dead, and by September 19, 1955, 90 per cent of the vines of all plots were dead.

Farm B, 1955

The yields obtained from the late harvest on Farm B were about 100 bushels per acre higher than the yields from comparable treatments at Farm A, reflecting both the better moisture-holding capacity of the soil and the higher level of available soil nitrogen at Farm B.

No significant yield response was obtained from more than 90 pounds of nitrogen per acre applied in the row at planting time. The late harvest yields from the plots which received 6 and 4 foliar applications of 7.5 pounds of nitrogen each in addition to 105 and 120 pounds of nitrogen in the row respectively, and the treatment which received 30 pounds of nitrogen as a side-dress in addition to 120 pounds in the row, were slightly larger than yields from plots which received only 90 pounds in the row at planting time. The lowest yield was obtained on plots which received 60 pounds of nitrogen in eight weekly foliar applications of urea. The yield differences were not statistically significant.

With the early harvest, all treatments which received additional nitrogen, regardless of method of application, had lower yields than those obtained from only 90 pounds of nitrogen per acre applied in the row at planting time. Early-harvested plants which had received an additional 45 and 60 pounds of nitrogen in weekly foliar applications produced the lowest yield.

The vines of all plots were green and vigorous in late July and early August. Prior to the heavy rains of mid-August, the foliage of the side-dressed plots (treatment 5) appeared more vigorous than the foliage in all other plots. After the heavy rains the foliage in all plots declined rapidly in color and vigor. By August 30, the foliage of treatment 1 was lightest green. On September 15 it was estimated that only 60 per cent of the vines of all plots were alive and on September 19, the percentages of vines still alive were 15, 29, 20, 27, 29 and 27 per cent in the plots of treatments 1 through 6 respectively. On September 29 the vines of all plots were nearly dead.

Early *vs.* Late Harvest — 1955

A study was made to determine the effect of deferred application of nitrogen by foliar application on tuber development. The vines in half of each plot were pulled on September 18, 1955. Those not pulled were 70 to 80 per cent dead on September 19. By September 29, 97 to 100 per cent of the vines were dead.

Despite the rapid decline of the vines, highly significant increases in yields of 23 to 86 bushels per acre were obtained from the late harvest as compared with those obtained from the early harvest as shown in table 3.

Yield increases from late-harvest over early-harvest on plots of treatments 2, 3, and 4, which received foliar applications, compared very favorably with the yield increase obtained from the 30 pounds nitrogen

side-dress treatment, and were larger than the yield increases obtained from the broadcast treatment, or from the treatment which received only 90 pounds of nitrogen per acre in the row.

The data in general suggests that greater tuber development occurred between September 19 and September 29 on plots which received more than 90 pounds of nitrogen. The data also indicate that additional applications of nitrogen, regardless of the method would result in lower yields if the growing season terminated early. If the season were long enough, it might be expected that 4 or 6 foliar applications of urea would compare favorably with a comparable amount of nitrogen applied as a side-dressing.

The Effect of Foliar Applications of Urea on the Nitrogen Content of the Leaflets

1954 — The nitrogen content of leaflets sampled periodically from plots at both locations in 1954 which received 60 pounds of nitrogen as eight foliar applications in addition to 90 pounds nitrogen per acre applied in the row, and of leaflets from plots which received only 90 pounds of nitrogen per acre in the row, are summarized in table 4.

The nitrogen content of the washed leaflets from the sprayed plots was higher than those from the unsprayed plots at both locations, especially on the last sampling dates, indicating that foliar applications of urea were effective in increasing the nitrogen content of the leaflets.

The higher nitrogen content of the leaflets obtained from Farm B correlated with the higher yields from this farm as compared with those from Farm A.

1955 — Leaflets were sampled periodically from all treatments in 1955. Regardless of the method of nitrogen application, the leaflets from plants which received additional nitrogen were higher in nitrogen content than leaflets from plots which received only 90 pounds of nitrogen in the row as shown in table 5 and figure 2.

Leaflets obtained on the last two sampling dates from the plots which received foliar applications of urea were slightly higher in nitrogen content than those from the plots which received side-dressed or broadcast applications of nitrogen. At both locations leaflets from plots which received 30 pounds of additional nitrogen as 4 foliar applications in addition to 120 pounds of nitrogen in the row, had a higher nitrogen content than leaflets from any other treatment, especially on the last two sampling dates.

The highest nitrogen content of the leaflets on Farm B occurred in the August 16 sampling, the nitrogen content of the leaflets then decreased progressively to the last sampling date.

The nitrogen content of the leaflets from Farm A was lower on 8/9/55 than on either the first sampling date, or two weeks later as shown in table 5. The lower nitrogen content may have been caused by a dilution effect of the plant tissue nitrogen as a result of the rapid growth due to ample moisture and lower temperature following the extended dry period which preceded the first sampling date.

The nitrogen content of leaflets sampled on comparable dates for comparable amounts of nitrogen applied were slightly higher in 1955 than in 1954 at both locations. The yields, however, were higher in 1954. It is reasonable to presume that had weather conditions permitted a longer growing season, larger yields would have been obtained in 1955.

Effect of Nitrogen Applications on Tuber Quality

The specific gravity of tubers from both locations in 1954 and 1955 are given in tables 1, 2, and 3. Method or rate of nitrogen application, time of harvest, and location had little or no effect upon specific gravity. The specific gravity readings on tubers from plots which received 60 pounds of nitrogen in eight foliar applications in addition to 60 pounds in the row in 1954 as shown in table 1 or in addition to 90 pounds of nitrogen per acre in the row on Farm B in 1955 as revealed in table 3, were slightly lower than the specific gravity of tubers from other plots. This was probably caused by the fact that the tubers were less mature. (5)

The lower specific gravity of the 1955 tubers as compared with the specific gravity of the 1954 tubers is correlated with conditions less favorable for maturity of the 1955 crop.

Hollow-Heart

Tuber samples from both locations in 1954 were practically free from hollow-heart. To determine the effect of treatments on the incidence of hollow-heart in 1955, 144 tubers from each treatment from each location were cut and inspected. On Farm A twenty hollow heart tubers were found. On Farm B, there were 24 and 41 hollow-heart tubers from the early- and late-harvests, respectively. No correlation of hollow heart incidence with nitrogen treatments was found.

After Cooking Darkening

In 1954 eight tubers from three replications of treatments 1, 2, 3, and 6 were tested for after cooking darkening. The percentage of tubers with after-cooking darkening was calculated on the basis of the total number of tubers examined from each experiment.

Tubers from Farm A showed more darkening (47 per cent) than those from Farm B (9 per cent). Tubers from plots which received 90 pounds of nitrogen in the row plus 60 pounds of additional nitrogen as eight sprays, were nearly free from the after-cooking darkening at both locations. The tubers from other treatments at both locations showed no such consistent pattern of the absence of after-cooking darkening.

Conclusion

The results showed that foliar application of urea is an effective means of supplying part of the nitrogen for Katahdin potatoes under Connecticut conditions. Foliar applications of urea can be used as a means of supplying additional nitrogen, when the plants are too large to be side-dressed..

No adverse effects upon tuber quality such as hollow heart incidence or after-cooking darkening were found correlated with foliar applied nitrogen from urea.

Foliar applications of nitrogen applied late in the growing season may tend to retard maturity of the crop, and result in lower yields, and tubers of slightly lower specific gravity in the event of a shorter than normal growing season.

The results obtained under the conditions of these experiments indicate

that the amount applied by foliar application should not be more than 30 to 45 pounds of nitrogen per acre in not more than 4 to 6 weekly sprays, with sufficient nitrogen provided at planting time to prevent nitrogen deficiency before all the nitrogen spray applications can be safely applied.

Literature Cited

1. Association Official Agricultural Chemists, Official and Tentative methods of analysis. Ed. 7. Washington, D. C. 1950.
2. Axley, J. H. and H. B. Winant. 1952. Better potato yields in western Maryland. Better Crops with Plant Food. 36: 6-9.
3. Hawkins, Arthur. 1946. Rate of absorption and translocation of mineral nutrients by potatoes in Aroostook County, Maine, and their relation to fertilizer practices. Jour. Amer. Soc. Agron. 38, No. 8, Pp. 674-675.
4. Lunt, H. A., C. L. W. Swanson, H. G. M. Jacobson. 1950. The Morgan soil testing system. Conn. Agr. Exp. Sta. Bull. 541.
5. McCall, W. and J. F. Davis. 1953. Foliar applications of plant nutrients to crops grown on organic soils. Mich. Agr. Exp. Sta. Quar. Bull. 35 (3): 373-383.
6. Prince, F. S., P. T. Blood, W. H. Coates and T. G. Phillips. Experiments with potatoes. New Hamp. Agr. Exp. Sta. Bull. 324. p. 38.
7. Shallock, D. A. and C. R. Skogely. 1952. Nitrogen sprays on potatoes. The R. I. Exp. Sta. Quar. Review, Res. 3: No. 1: 3-5.
8. Terman, G. L., Arthur Hawkins, C. E. Cunningham and R. A. Struchtemeyer. 1951. Rate, placement and source of nitrogen for potatoes in Maine. Maine Agr. Exp. Sta. Bull. 490.

POTATO QUALITY X. POST HARVEST TREATMENT TO PREVENT AFTER COOKING DARKENING.[1]

ORA SMITH[2]

One of the most widespread undesirable qualities of potatoes is the tendency for the tubers to turn dark after cooking. It appears first and to the greatest degree just under the skin at the stem end of the potato and decreases in intensity toward the apical end. The color may range from normal white of the unaffected tubers through shades of gray to almost black. It greatly detracts from the appearance of the cooked potato and renders it much less desirable. It has no known effect on flavor or nutritive value of the potato. Since many food products are accepted or rejected on the basis of color and appearance this defect undoubtedly has had a marked effect on the gradual decrease in potato consumption. It has been reported from practically every potato growing area in the world and has been the subject of investigation for over 50 years.

GROWING CONDITIONS AND DARKENING

Effect of Fertilizers. Many years ago it was reported that potatoes grown with a suboptimum supply of potassium would darken while those with potassium added remained white. Sulphate forms of potassium have been found to be better than the chloride or kainite forms for preventing discoloration. Jacob (9) found that the addition of potassium which decreases the amount of nitrogen in the potato also tends to decrease the darkening.

The content of nitrogen and potassium in tubers is closely related to the amounts of these substances which were added to the soil. Hansen (6) believes there is no direct connection between the content of potassium and darkening although darkening increases with the content of non-protein nitrogen compounds. Several investigators have shown that addition of nitrogen fertilizer in connection with lack of potassium, in other words a large N/K ratio, increases the tendency to darken.

Wallace and Wain (43) think that lack of phosphoric acid can result in darkening and that it is connected with the content of iron; with low phosphoric acid or a lack of potassium, iron would accumulate in the tubers. According to Tedin, *et al.* (37) darkening is associated with high content of protein and low amide N in tubers.

Soil. Reaction of the soil has an influence on the tendency of potatoes to darken, low pH soil is likely to increase darkening (Smith, 27, 28; Smith and Nash, 29, 30). These investigators also showed that potatoes grown in muck soil seldom darkened whereas those grown in the same area in mineral soils tended to discolor. They attribute these differences, however, to maturity and other factors that usually are associated with these soils.

Temperature. There is a relation between darkening of potatoes and the temperatures during the growing season, especially during the last

[1]Accepted for publication December 9, 1957.
Paper No. 415. Department of Vegetable Crops, Cornell University.
[2]Professor, Department of Vegetable Crops, Cornell University, Ithaca, New York.

several weeks of growth. Smith and Nash (31) and Smith, *et al* (32) showed that potatoes which were still alive and functioning for the last several weeks at temperatures under 50° F. had a much greater tendency to darken than those ripening at temperatures above 60° F. This is the primary reason for most potatoes harvested late to darken more than those which were harvested earlier when temperatures were higher. Potatoes from plants which have been killed early with chemicals also darken to a lesser extent than those killed later (D.D.S.F., 3; K. 11).

Soil Moisture and Light. Plants which are irrigated and heavily fertilized produce tubers with a greater tendency to darken than plants which receive only normal rainfall (Nash and Smith, 17; Smith and Nash, 29). In Scotland there is a higher degree of darkening in wet, cloudy summers than in dry, sunny summers (McIntosh, 13). On the other hand, in Denmark irrigated plants produced tubers of less darkening than those non-irrigated (Juul, 10). Shading plants increased the darkening of tubers (Nash and Smith, 17; Smith and Nash, 29).

Probably neither of the factors, light intensity nor soil moisture, directly affects the darkening tendencies. For instance, if soil moisture is low and it reduces the availability and absorption of certain fertilizer elements such as nitrogen or potassium it may decrease the prevalence of darkening by hastening maturity at a time when temperatures are relatively high. On the other hand, soil moisture may be high but the soil fertility very low and relatively little nitrogen or potash available, resulting in early maturity, during warm weather, and resultant decrease in darkening.

Varieties. Most European workers think that there are no pronounced differences between varieties in their tendency to darken (Hansen, 6; Parker, 20; V. S., V. V. S., 41). Nash (18), however, found marked differences in darkening between varieties grown under the same environmental conditions. In breeding experiments Rieman, *et al*, (22) crossed plants of white-boiling with those of dark-boiling potatoes and found that the tendency to remain white after boiling was totally or partially dominant.

Effect of Storage on Darkening

There seems to be a tendency for darkening to increase in potatoes as they are stored from fall to spring. Within the range of commercial storage temperatures this factor has no influence on darkening (Tottingham, *et al*, 40; K. 12). Smith, *et al*, (32) showed, however, that holding potatoes at high temperatures, such as 100° F. for 3 days greatly reduced the darkening or prevented it entirely.

Theories on Causes of Dark Color

Some investigators have assumed that the discoloration is a result of the tyrosine-tyrosinase-melanin reaction. As early as 1905, however, it was shown that there could be no connection between darkening and tyrosinase activity (Ashby, 1). Later workers (Merkenschlager, 15, 16; Tinkler, 39) state that tyrosinase could be active in this reaction during the short time before the cooking water reaches the boiling point. Oxidation is necessary for the formation of the dark pigment. It is assumed that the potato tissue

contains sufficient oxygen for the initial stages of the reaction but atmospheric oxygen is necessary for the final steps in oxidation. Boiling potatoes in a nitrogen atmosphere does not decrease the tendency to darken although those exposed to nitrogen after boiling do not discolor (Nutting & Pfund, 19). The potato cell organization is destroyed at 55° C. and tyrosinase is inactivated at 70° C. so that the reaction may take place between these two temperatures (Hansen, 6).

According to Tinkler (39) iron in the tubers increases darkening either by a catalytic action or the result of formation of an iron-phenol compound which is dark colored.

Iron Content of the Potato

In the literature one finds most varying reports on the content of iron in the potato. It can most likely be taken as certain that the iron content on an average varies between 2.5 and 10.5 mg. per 100 grams dry substance.

Only little is known of the form in which iron is found in the potato. Shackleton and McCance (25) report that 90 to 100 per cent of the iron is found in inorganic compounds and Shive (26) is of the opinion that the major part is found in insoluble form, most likely in the form of precipitated organic ferric complexes. There is probably an equilibrium between ferrous ions and ferric ions, of which the ferrous ions are the physiologically active.

That the darkening does not take place until after the boiling must be due to the fact that ferric ions are not present in the potato when the cell organization is destroyed as otherwise these ions in spite of the presence of ascorbic acid would form the dark colored iron-diphenol compound before the air was admitted. The ferric ions must, therefore, be present in an insoluble state or, at least, they are precipitated during the heating.

There is disagreement among the different investigators as to the influence of iron on darkening, but this may be due to the fact that in most cases the content of iron is determined while the important thing is the content of the free ferrous ions. Furthermore, the variations in the content of iron may have been so small that other factors which influence darkening may have concealed the influence of the iron. These might be such factors as hydrogen ion concentration or the ratio between the concentrations of o-diphenol and other complex formers.

If we assume that the darkening is due to the production of an iron-o-diphenol compound it should be expected that variations in the amount of ferrous ions present will influence the degree of darkening and certain circumstances point in that direction.

Content of O-diphenol in Potatoes

It is believed that the o-diphenol compounds are predecessors of the pigment that darkens potatoes and that the pigment is formed by oxidation. Smith, Nash and Dittman (32) found that darkening was produced by boiling potatoes in a solution of potassium bromate, an oxidizing agent, whereas a solution of stannous oxalate, a reducing agent, prevented darkening.

The most complete investigation in this field has been done by Juul (10). He found that caffeic acid and chlorogenic acid are present in the potato in approximately the same molar concentration. They form the major part of the o-diphenol content of the potato. He also found higher amounts of o-diphenol in the basal than in the apical ends of tubers although the differences were not always considered significant statistically.

It has been mentioned that darkening is worse in plants that were heavily fertilized with nitrogen. O-diphenol content usually is higher in tubers from plants which received large applications of nitrogen as compared with those with less nitrogen or with a low N/K ratio. Some of these differences, however, are not considered significant.

Darkening usually is less in immature than in mature tubers. Juul was not able to find any consistent correlation betwen o-diphenol content of basal ends of tubers harvested at different stages of maturity and color of the boiled potato.

Ross, *et al*, (23) found a high correlation between the o-diphenol content of tubers and darkening after boiling.

Effect of pH of Tuber Tissue

This discoloration always appears first and to the greatest extent and, in most cases, exclusively in the stem ends of tubers. Likewise the pH values of the stem ends of potatoes are higher than those of the apical portion. Potatoes boiled in acidulated water remain white rather than turning dark.

Smith, Nash and Dittman (32) showed that darkening depends on hydrogen ion concentration. By boiling potatoes in many solutions ranging from pH 3 to 9 it was found that darkening could be prevented in solutions more acid than the normal potato and intensified at reactions more alkaline than that of normal potatoes. The pH of tubers also was changed by storing them in various gas mixtures. Any storage treatment which resulted in decreased pH of the tubers also decreased or prevented darkening. At high temperatures (100° F.) or in inert gas (nitrogen) anaerobic oxidation is increased. This results in the formation and accumulation of organic acids which lower the pH of the tubers. Because of the low supply of oxygen in these tissues the o-diphenol compounds may be present in the reduced form and further oxidation to dark colored compounds would be unlikely. Further work by Smith and Kelly (33) with potatoes treated with ethylene chlorhydrin to greatly increase respiration rate followed by storage in various gases at several temperatures showed that there is not a perfect relationship between pH of the tuber and appearance of blackening, without regard to the method of changing the pH of the tuber.

Wager (42) thinks that the quantity of darkening is independent of the degree of acidity of the potato, but depends on the amount of pigment, the color of which changes reversibly with pH. The pigment from dark boiling potatoes did not change in quality between pH 1.5 and 7.5. The intensity of the color decreased rapidly from pH 9.5 to pH 6.5 and then slower as the pH decreased further.

Juul (10) also states that darkening depends on the hydrogen ion concentration of the potato; there is a linear relationship between pH and color. Darkening always is greatest in the stem end of the potato and pH

always is highest in this same area. The pH of the potato increases with the degree of ripeness (Juul, 10). Juice from dark boiling potatoes has a higher pH than juice from those which remain white. The hydrogen ion concentration of the boiled potato is between pH 5.7 and 6.4. In the raw potato the pH is lower because of the presence of CO_2 in the juice.

Potato juice has the least buffer capacity at the natural pH. The buffer index curve increases in both the acid and the alkaline direction. Between pH 10 and 8 the buffer material is mainly amino acids and asparagine. From pH 8 to 3, it is citric acid and maleic acid in addition to amino acids and asparagine. Between pH 6 and pH 3 there is also a weak phosphate buffer effect (Guthrie, 5).

Except citric, tartaric and partly maleic acid most organic acids lose their buffer capacity in the region pH 5.4 to 6.2 and the phosphate and carbonate systems have just started to have some effect. This means that carbonic acid will be of great importance. The pH of potato juice with 20 per cent CO_2 in the air above the juice changed from 6.23 to 5.93 and in 100 per cent CO_2 it changed to pH 5.56. The increase in pH of juice from potatoes which had been treated with ethylene chlorhydrin was due primarily to a decrease in citric acid content. The apical ends of potato tubers have the lowest pH and also are highest in organic acids (Prunet, 21).

(a) *Influence of Fertilizer on the Buffer Capacity of Potato Juice.*

The ratio of nitrogen to potash in a fertilizer for potato production influences the hydrogen ion concentration of the potatoes. This ratio may also influence the content of organic acids in potatoes and therefore affect their buffer capacity. Above pH 8, potatoes having the greatest buffer capacity were fertilized only with nitrogen (Juul, 10). They also have an increased content of amino acids. Below pH 8 and especially between pH 6.5 and 5.0 the buffer capacity is decreased. The decrease in buffer index is not as marked below pH 5.0, especially in the most acid region.

(b) *Influence of Fertilizer on the Content of Citric Acid of the Potato.*

Citric acid concentration varies between 0.3 and 1.0 mg. per 100 ml. of juice. (Hartman and Hillig, 7; Thunberg, 38; Steinhardt, 36). In the region pH 5.4 to 6.2 there is a difference in the buffer capacity of potato juice from tubers grown with nitrogen only and those with nitrogen and potash. This change in buffer capacity may be explained by the change in citric acid concentration (Juul, 10).

Maleic acid is of little importance in this connection since it occurs in only 1/20 the amount of citric acid (Curl and Nelson, 2).

Importance of Iron and O-diphenol Compound in Blackening

Juul states that undoubtedly an iron compound in the potato is involved in the reaction which results in after cooking darkening. He found that the violet ferric-dipyrocatechol compound may be decolored by pyrophosphate. He cut potatoes in half longitudinally, boiled one set in 10 per cent pyrophosphate solution and the opposite halves in tap water. Reactions of the solutions was pH 6.33, therefore, color improvement could not be due to lowering pH. In every case pyrophosphate repressed the

darkening completely. Moreover, decoloring with pyrophosphate was made after boiling by leaving the potatoes in a pyrophosphate solution for some time. Recently Smith (35) treated unpeeled whole potatoes by dipping them in a solution of sodium acid pyrophosphate and also by vaporizing the chemical in the air surrounding the potatoes. After 48 hours in a tight container treated potatoes showed little or no darkening after boiling, whereas, untreated potatoes exhibited it to a great extent. This apparently results in the formation of colorless iron-pyrophosphate complexes and a consequent white potato.

It is not possible from these data to conclude anything as to the kind of iron compound involved.If we assume that it is an o-diphenol-iron compound it should be possible to repress the darkening by boiling the potato in a substance which forms specific compounds with o-diphenols without simultaneously binding the iron. Such a substance is boric acid.

Pyrocatechol, an o-diphenol, reacts with boric acid to form dipyrocatechol-boric acid (Hermans, 8; Meulenhoff, 14). The ferridipyro-catechol compound can be discolored by using a sufficiently strong concentration of boric acid. Juul conducted such experiments and found that boric acid decolored the o-diphenol complex considerably because boric acid bound part of the o-diphenol. Similar results were obtained with caffeic acid. pH in all cases was buffered to 6.50.

The possibility that it was iron which had made a complex with boric acid is very likely particularly at the concentrations which were used. Very little is known about the boric acid complexes of iron, but they are undoubtedly much weaker than the o-diphenol compound mentioned above.

If the darkening is due to an o-diphenol, it should be possible to decolor dark boiled potatoes by placing them in a boric acid solution. Juul placed longitudinal halves of potatoes, one half hour after boiling, in tap water and the opposite halves in 1 per cent boric acid solution. Both were buffered to pH 6.4. After one half hour they were removed and it was found that boric acid effected a decrease in darkening.

Juul has shown that darkening of the potato is due to an iron-o-diphenol compound and that the o-diphenol is a mixture of caffeic and chlorogenic acids. He has concluded that the darkening process of the potato after boiling depends on the following factors:

(1) pH. The complexity of the formed iron-caffeic acid compounds increases strongly with pH. Therefore, the darkening will be increased under such growing conditions which increase pH. It should be emphasized that this increase in pH refers to boiled tissue or juice. When no such relation was found in some experiments it may be due to the fact that these things were not taken into account in the measurements made. Varying amounts of carbon dioxide and ammonia in the raw juice may have influenced the results.

(2) Iron Concentration. The concentration of ferrous ions is important as it increases the amount of pigment.

(3) Concentration of Caffeic and Chlorogenic acids relative to the concentration of other iron-complex-forming ions of importance. Certain complications exist since simultaneous changes of concentration of these ions may take place in the same or in opposite directions.

Juul also states that in the same locality changes in pH probably are

of greatest importance while color differences between potatoes from different localities may be due to one of the other factors or perhaps to both of them.

Effect of Irradiation on Darkening

Sawyer (24) irradiated Cobbler, Green Mountain, Katahdin and Russet Burbank tubers with gamma rays at dosage levels of 5000, 7500, 10,000 and 12,500 r. Tubers from each of these as well as untreated were boiled after 8 months storage at 50° F. Dosages of 10,000 and 12,500 r developed worse after cooking darkening than the checks or lighter dosages.

In another experiment Katahdin tubers were treated with gamma and fast electron irradiation at dosages of 10,000, 20,000, 40,000 and 80,000 r. After 10 months storage at 50° F. it was found that the two highest dosages gave an increase in after cooking darkening when compared with the untreated. The blackening was more pronounced with gamma than with fast electron irradiation.

Recent Methods to Prevent After Cooking Darkening

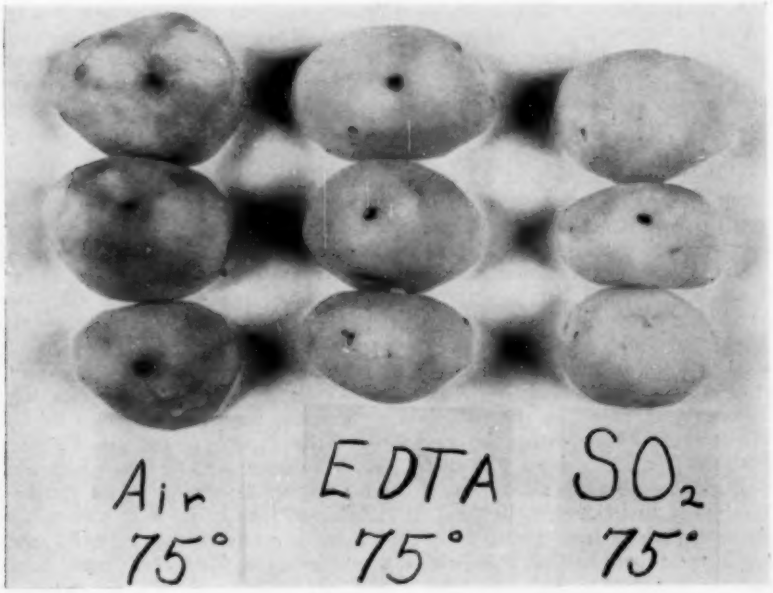
Since the most logical theory on the cause of after cooking darkening appears to be the reaction of certain types of o-diphenols and certain forms of iron, our recent experiments have emphasized this phase. The ferrous ions of the tuber combine with an o-diphenol giving a colorless or faintly colored compound. This compound oxidizes when exposed to air forming the deeply colored ferric compound. Juul was able to prevent after cooking darkening of potatoes by boiling them in a weak sodium pyrophosphate or sodium acid pyrophosphate solution. This resulted in the formation of colorless iron-pyrophosphate complexes and consequently white boiled potatoes.

It occurred to us that we might be able to prevent after cooking darkening by sequestering or chelating the iron in potatoes. A sequestering agent is a compound that will inactivate a metallic ion by forming a water soluble complex in which the metal is held in a non-ionizable form. A chelating agent is a compound which inactivates a metallic ion by making it an integral part of an inner ring structure. Since ethylene diamine tetraacetic acid (EDTA) and its salts chelate iron preferentially to all other commonly occurring metals it was first selected for trial. Results of work of Smith and Muneta (34) and Greig and Smith (4) show that after cooking blackening can be reduced or prevented by spraying chelating chemicals on plants in the field and by dipping peeled potatoes in dilute solutions 24 hours or more before boiling. Other chemicals such as gluconic acid, citric acid, sodium gluconate, sodium citrate, ammonium gluconate and sodium bisulfite also reduced after cooking darkening when applied as a spray to foliage in the field. It is believed that in most instances the above chemicals have reduced darkening by sequestering or chelating the ion in the tubers so that it is held in a non-ionizable form and cannot take part in a reaction with o-diphenol and, therefore, prevents the normal formation of the dark colored pigment, a ferric o-diphenol compound. During the past year we have tested a number of chemicals to determine their effect on after cooking darkening by treating the unpeeled whole potatoes after several months storage.

Materials and Methods

Katahdin tubers harvested in September and October and stored at 40° F. until July and August were used. Treatments consisted of 1, SO_2 gas, 2, several volatilized or evaporated chemicals in which potatoes were confined for 48 hours and 3, several solutions in which tubers were dipped for 2 minutes. All treatments consisted of placing potatoes in an airtight container for 48 hours after treatment or during treatment at temperatures of 50° and 75° F. Potatoes were then removed from the containers, peeled and boiled in the usual manner and after one-half hour observed for darkening. Photographs of the stem ends of potatoes were made within three hours of cooking. Figures 1, 2, 3 show the effects of the various chemicals on darkening.

No darkening appears in tubers treated with sulfur dioxide gas. These figures also show that there was little or no darkening in those potatoes which were treated with sodium bisulfite, sodium gluconate, sodium acid pyrophosphate and Versenol (trisodium salt of N-hydroxyethylethylene-diaminetriacetic acid) (HEDTA). (Tables 1 and 2). Versenol forms ferric chelates with the trivalent or ferric iron ions. The proper concentration and the time and temperature during treatment have not been worked out yet.



1 2 3

FIGURE 1.—After cooking darkening of potatoes (1) untreated, (2) dipped in solution of NaHEDTA and (3)treated with sulfur dioxide gas for 48 hours. All were held in a tight container at 75° F. during or following treatment.

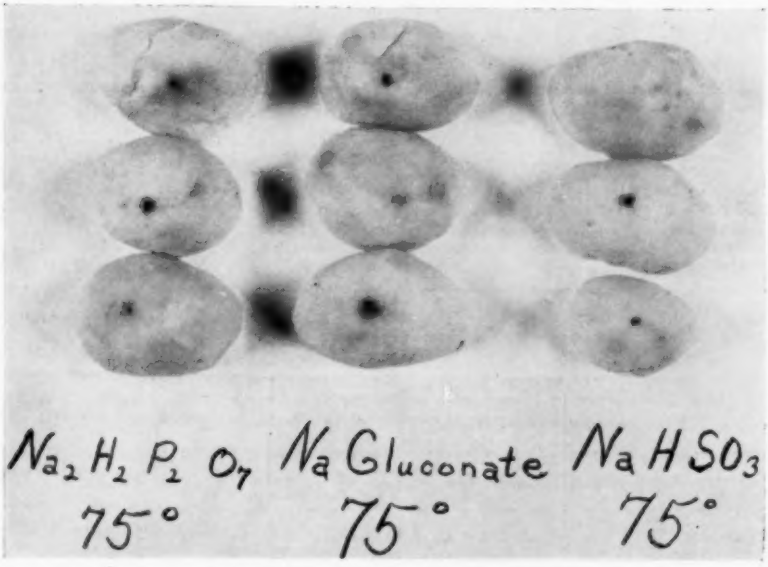


FIGURE 2.—After cooking darkening of potatoes (1) dipped in sodium acid pyrophosphate solution, (2) dipped in sodium gluconate solution and (3) dipped in sodium bisulfite solution. All were held in a tight container at 75° F. following treatment.

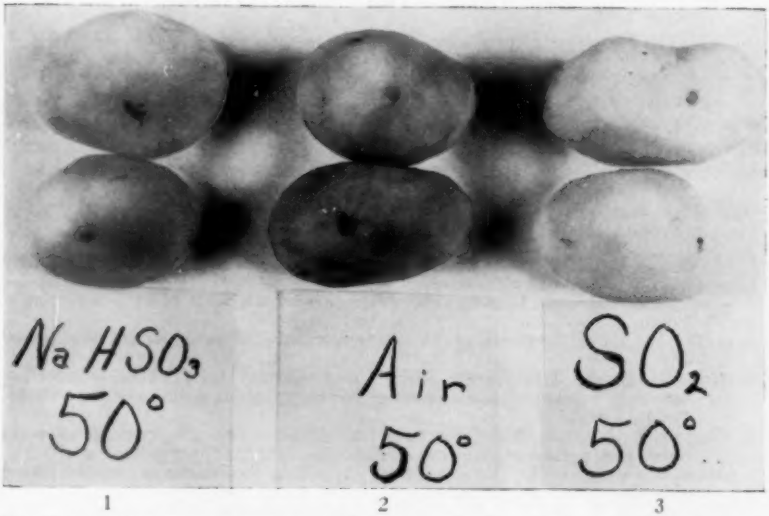


FIGURE 3.—After cooking darkening of potatoes (1) dipped in sodium bisulfite solution, (2) untreated and (3) treated with sulfur dioxide gas for 48 hours. All were held in a tight container at 50° F. during or following treatment.

TABLE 1.—*Effect of chemical treatments of whole unpeeled potatoes on after cooking darkening (Stored at 75° F.)*

Chemical and Concentration (2 per cent)	Color Rating*
Untreated	6
Sodium gluconate	9
Sodium acid pyrophosphate	9
Sodium bisulfite	9
Versenol	10
Sulfur dioxide	10

*10 = White, no darkening, with progressively lower numbers indicating increasing amount and intensity of discoloration.

TABLE 2.—*Effect of chemical treatments of whole unpeeled potatoes and of holding temperatures on after cooking darkening.*

Chemical and Holding Temperature	Color Rating*
Untreated, 50° F.	5
Untreated, 75° F.	6
Sodium gluconate, 75° F.	9
Sodium bisulfite, 50° F.	10
Sulfur dioxide, 50° F.	10
Sulfur dioxide, 75° F.	10

SUMMARY

Results of these preliminary experiments indicate that after cooking darkening can be reduced or prevented by treating whole unpeeled potatoes with several chemicals after harvest. Concentrations, length of treatment and temperature during treatment need to be worked out more completely.

LITERATURE CITED

1. Ashby, S. F. 1905. A contribution to the study of factors affecting the quality and composition of potatoes. Jour. Agr. Sci. 1: 347.
2. Curl, A. L. and E. K. Nelson. 1940. The non-volatile acids of the potato. Amer. Potato Jour. 17:328.
3. D.D.S.F. 1939. Beretning nr. IV om forsogs-arbejdet pa selskabets forsogsgard 37.
4. Greig,W. S. and Ora Smith. 1955. Potato Quality IX. Use of sequestering agents in preventing after cooking darkening in pre-peeled potatoes. Amer. Potato Jour. 32:1.
5. Guthrie, J. D. 1934. Metabolism of citric, sulphuric and nitric acid in the potato tuber. Contr. Boyce Thompson Inst. 6: 247.
6. Hansen, Frode. 1935. Aarsagerne til Kartoflers Morkfarvning ved Henstand efter Kogning, Kobenhavn.
7. Hartman, B. G. and F. Hillig. 1934. Acid constituents of food products. Jour. Assoc. Off. Agr. Chem. 17: 522.
8. Hermans, P. H. 1925. Uber die Konstitution der Borsaure und einiger ihrer Derivate. Z. anorg. Chem. 142: 83.

9. Jacob, A. 1935. Der Einfluss der Kalidungung auf die chemische zusammensetzung der ernteprodukte. Pflanzenernahr. W. Dungung 37: 1.
10. Juul, F. 1949. Studier over kartoflens morkfarvning efter Kogning. I. Kommission hos Jul. Gjellerups Forlag. Kobenhavn. 154 pp.
11. K. 1945a. Landbo-og Husmandsforeningernes Forsogsvirksomhed i Planteavl. 20-21. Meddelelse.
12. K. 1945b. Beretning om Landboforeningernes Virksomhed for Planteavlen paa Sjaelland: 188.
13. McIntosh, T. P. 1942. Cooking quality of potatoes. Scottish Jour. Agr. 24: 38.
14. Meulenhoff, J. 1924. Bijdrage tot de kennis van organische Boriumverbindungen. Dis. Delft.
15. Merkenschlager, F. 1929a. Uber das Schwarzwerden der Kartoffelkollen. Nachrbl. deut. Pflanzenschutzdienst 9: 20.
16. ————. 1929b. Das Schwarzwerden der Kartoffelkollen eine Kalimangelerscheinung Ernahr. Pflanze 25: 275.
17. Nash, L. B. and Ora Smith. 1939. Potato Quality II. Relation of mineral nutrition and alterations in light intensity to cooking quality. Proc. Amer. Soc. Hort. Sci. 37: 861.
18. ————. 1941. Potato Quality IV. Relation of variety and environmental conditions to partial composition and cooking quality. Amer. Potato Jour. 18: 91.
19. Nutting, H. W. and M. C. Pfund. 1942. Nature of darkening of cooked potatoes. Food Res. 7: 48.
20. Parker, W. H. 1932. Quality in potatoes. Chem. & Ind. 51: 94.
21. Prunet, A. 1892. Sur la constitution physiologique des tubercles de pomme de terre dans ses rapports avec le developpement des bourgeons. Compt. Rend. 114: 1079.
22. Rieman, G. H., W. E. Tottingham and J. S. McFarlane. 1944. Potato varieties in relation to blackening after cooking. Jour. Agr. Res. 69: 21.
23. Ross, A. F., W. E. Tottingham and R. Nagy. 1939. Characteristics of the tyrosinase system in potatoes which blacken after boiling. Plant Physiol. 14: 549.
24. Sawyer, R. L. 1957. Effect of irradiation on after cooking darkening. Unpublished data.
25. Shackleton, L. and R. A. McCance. 1936. The ionizable iron in foods. Biochem. Jour. 30: 582.
26. Shive, J. W. 1941. Trace elements in the nutrition of plants. Plant Physiol. 16: 435.
27. Smith, Ora. 1937. Some factors affecting culinary quality of potatoes. Amer. Potato Jour. 14: 221.
28. ————. 1940. Potato research at Cornell University. Amer. Potato Jour. 17: 27.
29. ———— and L. B. Nash. 1940a. Potato Quality III. Relation of soil reaction, irrigation and mineral nutrition to cooking quality. Proc. Amer. Soc. Hort. Sci. 38: 507.
30. ———— and ————. 1940b. Potato Quality I. Relation of fertilizers and rotation systems to specific gravity and cooking quality. Amer. Potato Jour. 17: 163.
31. ———— and ————. 1942. Potato Quality V. Relation of time of planting, time of harvest and fertilizer treatment to composition and cooking quality. Jour. Amer. Soc. Agron. 34: 437.
32. ————, ———— and A. L. Dittman. 1942. Potato Quality VI. Relation of temperature and other factors to blackening of boiled potatoes. Amer. Potato Jour. 19: 229.
33. ———— and W. C. Kelly. 1944. Potato Quality VII. Changes in potato tubers following treatments which affect blackening. Proc. Amer. Soc. Hort. Sci. 44: 384.
34. ———— and P. Muneta. 1954. Potato Quality VIII. Effect of foliar applications of sequestering and chelating agents on after cooking darkening. Amer. Potato Jour. 31: 404.
35. ————. 1957. Preventing after cooking darkening in potatoes. (Unpublished data.)
36. Steinhardt, B. 1945. Citric acid from raw potatoes. Kungl. Fysiograf. Sallskap. Lund. Forh. 15: 211.

37. Tedin, O, C. Brodderson, L. Aronson and E. Larsson. 1943. Sortsforsok med potatis i Skane och Blekinge 1939-1940. p. 65. Kristiansstad.
38. Thunberg, T. 1945. Der Citratgehalt der Kartoffel. Kgl. Fysiograf Sallskap. Lund. Forh. 15: 58.
39. Tinkler, C. K. 1931. The blackening of potatoes after cooking. Biochem. Jour. 25: 773.
40. Tottingham, W. E., R. Nagy and A. F. Ross. 1936. The problem of cause of blackening in cooked potatoes. Amer. Potato Jour. 13: 297.
41. Vereinigung Schweiz. 1947. Versuchs-und Vermittlungs-stellen fur Saatkortoffeln 11. Tatigkeitsbericht, p. 48.
42. Wager, H. G. 1945. The effect of pH on stem end blackening of potato. Biochem. Jour. 39: 482.
43. Wallace, R. and L. Wain. 1943. The blackening of cooked potatoes. Agriculture 50: 425.

A FUNGUS-LIKE STRUCTURE IN POTATO TUBERS AND POTATO TISSUE CULTURES[1]

DONALD A. YOUNG[2]

The potato is a crop plant grown extensively throughout the world for its starchy tubers which are used as human food, animal feed and for industrial purposes. The presence of contaminating bacteria and fungi in potato tissue has been reported by several workers.

Early evidence indicated that the fungi associated with potato roots were involved in tuber formation (2, 3, 5, 13). Various aspects of the relationship between mycorrhizal fungi and the potato have been reported (8, 16). The mycelium arising from a large soil borne spore common in Illinois soils was found by Gerdemann (9) to form an association with the roots of red clover, corn, strawberry and sweet clover. A similar association was formed with the roots of potato seedlings (Personal communication). Other investigations have shown that under certain growing conditions tubers were formed when mycorrhizal fungi were not present (6, 14, 15, 18). The presence of bacteria in healthy potato tubers has been reported (22, 26).

A mycorrhiza-like mycelium in potato tubers was reported by Cooper, Rieman and Hougas (7). The mycelium was present in healthy tubers from 30 varieties and 150 seedlings of *Solanum tuberosum* as well as *S. andigenum, S. demissum, S. gibberulosum, S. lanciforme, S. pinnatisectum, S. semidemissum* and *S. verrucosum*. A study of the development of the tuber revealed that the fungus came in contact with the growing tip of the rhizome, penetrated the apex and became associated with the vascular tissue. Shortly after penetration the apical portion of the rhizome enlarged to form a tuber. Examination of rhizomes which failed to tuberize did not reveal the presence of a fungus. In preliminary tests plants from true seed grown on a sterile substrate did not produce tubers.

The purpose of this research was to investigate further the nature and function of the mycorrhiza-like mycelium found in potato tubers.

MATERIALS AND METHODS

Freehand sections of tubers, roots, seeds, stems, rhizomes and tissue cultures were stained with rose-azurine, cotton blue and aceto-carmine. Potato rhizomes, tubers and tissue cultures were fixed in FAA, treated according to the standard paraffin technique and stained with safranin-fast green and Delafield's haematoxylin-safranin. Other potato tissue cultures were fixed in 6:3:1 Carnoy's fixative using 70 per cent alcohol, treated according to the standard paraffin technique and stained with Delafield's haematoxylin.

[1]Accepted for publication December 9, 1957.
Contribution 659 from the Department of Genetics, University of Wisconsin, Madison 6, Wisconsin. A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Wisconsin. Supported in part by the Research Committee of the Graduate School with funds from the Wisconsin Alumni Research Foundation.
[2]Research Officer, Experimental Farm, Fredericton, New Brunswick, Canada.

The original callus cultures were supplied as stocks from tissues isolated at Wisconsin (28). One culture was supplied by Dr. S. M. Caplin. In addition, callus cultures were subsequently isolated from potato tubers, stems, rhizomes, and leaf petioles and grown on an agar medium (25). An earlier study (29) indicated that potato tissue cultures contained a fungus-like structure. Bacitracin, aureomycin hydrochloride, sodium penicillin and terramycin hydrochloride were therefore added to the medium in an experiment designed to test the effect of antibiotics on the fungus and on the host tissue. Cultures were maintained on the antibiotic containing media for a period of three months through three transfers before examination.

Tubers were produced under aseptic conditions using a procedure described by Barker (1). Rapidly growing sprouts ¾ to 1 inch long were removed from tubers, surface sterilized, and placed in 25 x 200 mm. test tubes on White's agar medium supplemented with 2.5 mg. calcium pantothenate per liter. The sprouts were kept in the culture room for approximately four months. Tuberization was aided if the cultures were occasionally placed in a 5°C refrigerator for a day.

Potato plants were grown under aseptic conditions in 25 x 200 mm. test tubes, 500 ml. and 800 ml. Erlenmeyer flasks. Potato seeds, surface sterilized in 1/1000 $HgCl_2$ or a 0.5 per cent solution of sodium hypochlorite were placed on autoclaved washed white sand moistened with a modified 1 Hoagland's (24) nutrient solution. One group of plants was placed in the Wisconsin soil temperature tanks maintained at 16-18°C with an air temperature ranging from 21 to 24°C. Other plants were grown on a greenhouse bench with temperatures maintained at 19°C and 23°C. Additions of 1, 5 and 10 per cent glucose were made to the Hoagland's solutions in trials maintained at 19°C and 23°C. Plants were kept in the open sunlight supplemented with artificial light.

Experimental Results

The Fungus-like Structure in Potato Tubers

The tubers of 60 potato varieties, 185 seedlings and 45 *Solanum* species were examined during the course of the present study. These tubers were grown under normal conditions in the field and greenhouse in Wisconsin, several other states and Canada, and were collected and examined over a period of four years. A fungus-like structure was present in all tubers examined.

The mycelium was clearly evident at 100 magnifications in freehand section 50 to 60 microns thick stained with aceto-carmine (Fig. 1). In such a preparation the mycelium took a brilliant red stain, the potato nuclei a deep pink color and the remainder of the tissue was unstained. Other stains that demonstrated the fungus satisfactorily were those that stained the mycelium deeply and the plant parts faintly or not at all. Such a contrast aided in revealing the presence of the fungus. Cotton blue (aniline blue) in water, alcohol or FAA and rose-azurine in water stained the fungus and provided fair contrast between the mycelium and the tuber section.

Microtomed sections of tubers and enlarged rhizomes, ranging from 6 to 10 microns in thickness, were stained with safranin-fast green and

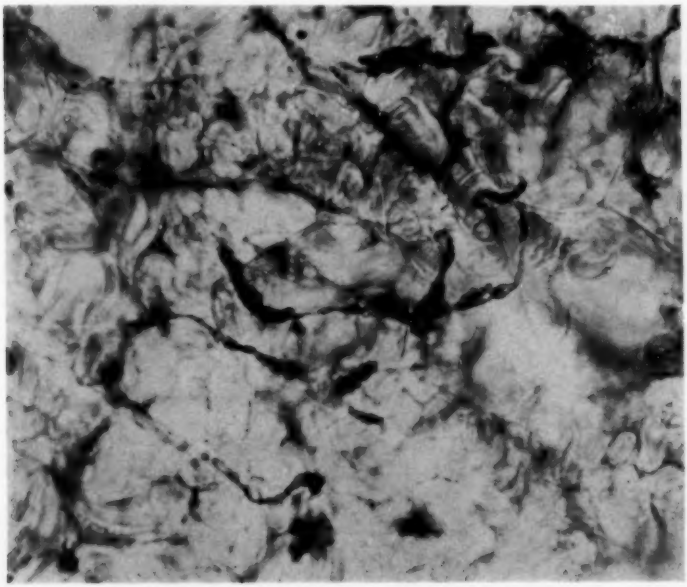


FIGURE 1.—Aceto-carmine stained freehand section of a tuber, var. Red Pontiac, showing the fungus.

Delafield's haematoxylin. In some cases material which resembled the fungus was present and this material could be followed in serial sections. However, in no case did the mycelium occur with the characteristics typically evident in freehand sections.

The fungus appeared as a branching inter- and intracellular hypha in properly stained freehand sections. Stains specific for cellulose and callose did not reveal hyphal walls or cross walls. Nuclei were not evident within the mycelium. The diameter of the hyphae varied from 0.9 to 7 microns. Single hyphal strands ranging up to 285 microns in length were present. Branching occurred, in general, from hyphae with a diameter of 4 or more microns and the branch was considerably smaller than the main hypha. Strands one micron in diameter and up to 70 microns long which had originated from a larger hypha were common. Such strands connected two main hyphae or the strand would become increasingly smaller in diameter and apparently terminated beyond the range of vision.

The fungus was present in potato tubers ranging in size from slightly enlarged rhizomes to large mature tubers. It was most abundant in tubers 4 mm. to 10 mm. in diameter. The hyphae were large and apparently in stages of active growth in such tubers. In larger tubers, and particularly in mature tubers which had been stored for a considerable time, the hyphae were widely dispersed and did not readily take the stain.

The fungus was present in all parts of the tubers although most common in the cortex and vascular areas (Fig. 2). It was considerably more abundant in the tissues forming the apical portion of the tuber than in those at the stem end. Oftentimes there was a close association between the fungus and the vascular tissue. Large hyphae were present adjacent to and inside of xylem vessels. Such hyphae often occupied a portion of a xylem vessel 15 microns long but did not appear to plug the vessel. Hyphae were observed to pass through parenchyma tissue from one xylem element to another. Occasionally the fungus was present in the periderm. When present in this tissue the hyphae were large in size, seldom branched and appeared to have penetrated the periderm from the exterior (Fig. 3). In tubers 2 to 5 mm. in diameter hyphae were abundant in the periderm and the cortex at the apical end of the tuber.

The Fungus-like Structure in Tissue Cultures

A mycelium, similar to that occurring in tubers, was present in tissue cultures derived from the tubers, stems, rhizomes and leaf petioles of potato plants (Table 1). The fungus occurred as branching hyphae and cell walls or cross walls were not demonstrated. The hyphae were generally intercellular, and branching was not as common as in tuber tissue. The mycelium was best observed in freehand sections stained with cotton blue or aceto-carmine (Fig. 4.). Microtomed sections stained with safranin-fast green or Delafield's haematoxylin did not reveal the fungus.

In tissue cultures the fungus was not as abundant and was more difficult to stain properly than in young tubers. However, it was present to varying degrees in each culture. Hyphae were nearly non-existent in the young, small-celled, meristematic regions of the culture but were present in the more mature regions of the culture piece. Difficulty was encountered in demonstrating the presence of the fungus in soft-type cultures. Such cultures were not firm enough for sectioning and it was necessary to spread and mash them. This process apparently broke up the mycelium and made it difficult to detect.

The mycorrhizal nature of the fungus was indicated by its relation to the tissue culture and the culture medium. The fungus in no case grew from the tissue culture onto the medium in the culture bottle. It failed to grow on yeast extract agar (*27*) or on Robbins' and Hervey's medium (*21*) which are commonly used to reveal contaminations in tissue cultures. No spores were observed in potato tubers or in culture materials.

Tissue cultures were established from the stems of potato seedlings grown under aseptic conditions. Surface sterilized seeds of the crosses (X131.52 x X141.52) and (B606 x X143.52) were planted in Erlenmeyer flasks on sterile sand moistened with 1 Hoagland's solution. When three inches high these seedlings were removed from the flask and tested for sterility on yeast extract agar. The stems were cut into pieces 1 to 2 cm. in length and placed on the Steward and Caplin (*25*) culture medium. After three transfers the cultures derived from the stems of eight plants were microscopically examined. The mycelium was present in cultures derived from five of the plants but was not observed in the remaining three.

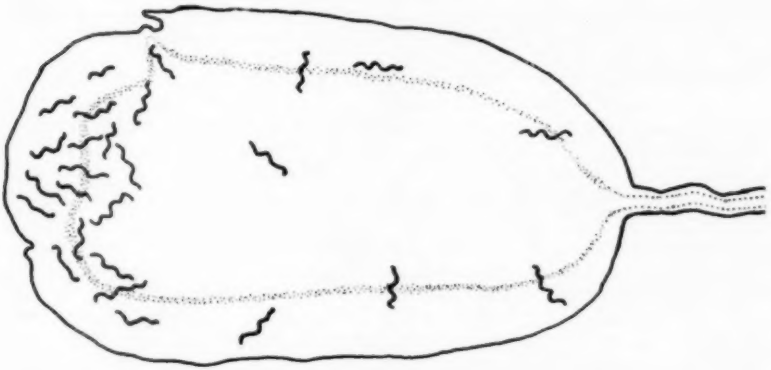


FIGURE 2.—Longitudinal section of a potato tuber 1.5 cm. in length showing the regions in which the fungus mycelium was most abundant.

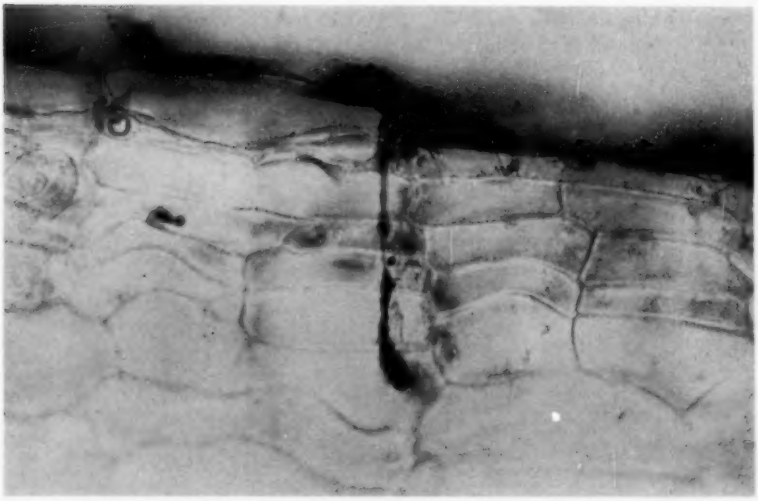


FIGURE 3.—Fungus mycelium in the periderm of a potato tuber, var. Chippewa.

TABLE 1.—*Potato varieties and seedlings from which tissue cultures harboring the fungus-like structure were established.*

Variety	Plant Portion
Antigo	Tuber
Chippewa (free from Virus X)	Tuber
Chippewa (infected with Virus X)	Tuber
Chippewa (infected with Virus X and Virus Y)	Tuber
Hindenburg	Tuber, pith
Hindenburg	Tuber, vascular region
Irish Cobbler	Rhizome
Irish Cobbler	Tuber
Irish Cobbler	Stem
Irish Cobbler	Leaf petiole
Katahdin	Tuber, pith
Kennebec	Tuber, pith
Kennebec	Tuber, vascular region
Kennebec	Tuber, cortex
Keswick (free from Virus X)	Tuber
Keswick (free from Virus X)	Stem
Red Beauty	Tuber, pith
Red Beauty	Tuber, vascular region
Red Beauty	Tuber, cortex
Russet Burbank	Tuber, vascular region
Russet Burbank	Tuber, pith
Russet Sebago	Tuber, vascular region
Russet Sebago	Tuber, pith
Wis. S4	Tuber
Wis. seedling (X143 x X125)	Stem
Wis. seedling (X141 x X137)	Stem
Wis. seedling (X131 x X141)	Stem
Wis. seedling (B606 x X143)	Stem

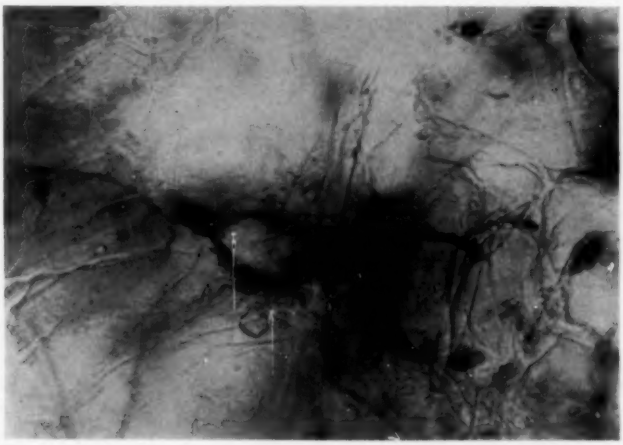


FIGURE 4.—Fungus mycelium in potato tissue culture derived from tuber tissue, var. Katahdin.

Tissue Cultures Free of the Fungus-like Structure

Tissue cultures were produced from small groups of cells by modifying the procedure of Muir *et al* (20). A cluster of approximately 20 cells was excised from the meristematic regions of established tissue cultures. The cells were placed on a 7 x 7 mm. square of sterile filter paper which had been resting for the three previous days on the surface of an actively growing 'host' culture. The filter paper with the small number of cells was then returned to its culture of origin. When the culture on the upper surface of the paper reached a diameter of 5 mm. it was placed on an agar medium. A total of 19 new cultures of the varieties Red Beauty, Hindenburg and Wisc. M804 (free of Virus X) were established in this manner. During succeeding transfers these cultures were examined microscopically. After four examinations covering a period of four months through four transfers, two cultures of Red Beauty, four cultures of Hindenburg and one culture of Wis. M804 appeared to be free of the fungus. The cultures were maintained for six months and a further examination did not reveal the fungus. There were no apparent differences, either in morphology or rates of growth, between cultures where the fungus was present and cultures free of the fungus.

Certain antibiotics influenced the mycelium growth in the host tissue cultures. Cultures of Russet Burbank and Red Beauty were grown for a period of four months on media containing four antibiotics (Table 2). After this incubation period no hyphae were present in Russet Burbank cultures grown on media containing 100 ppm. aureomycin hydrochloride, 50 and 100 ppm. sodium penicillin and 100 ppm. bacitracin. The fungus was present in all cultures of Red Beauty in all treatments. However, there appeared to be a decided reduction in fungal growth in several of the cultures containing antibiotic materials. In the case of Red Beauty cultures grown on 100 ppm. bacitracin, eight preparations for microscopic examination, instead of the usual single preparation, were required to reveal the presence of the mycelium.

Table 2.—*The effect of antibiotics in the culture medium on the fungus-like structure.*

Antibiotic	Concentration	Red Beauty Tissue Culture	Russet Burbank Tissue Culture
Bacitracin	100 ppm.	+*	—
Terramycin	25 ppm.	+	+
	50 ppm.	+	+
Aureomycin	50 ppm.	+	+
	100 ppm.	+	—
Penicillin	50 ppm.	+	—
	100 ppm.	+	—
Check		+	+

*+ Fungus present
— Fungus absent

Tubers Produced on Plants Grown under Aseptic Conditions

Plants grown under aseptic conditions from true seed were subjected to an environment quite different from that in which potato plants are normally grown. The stems of such plants were elongated, and a small simple, oftentimes rolled leaf developed at each node. Numerous small branching rhizomes were produced.

Three hundred and seventy-six plants were grown from true seed under aseptic conditions.

Eight of these plants produced tubers. One plant produced tubers in a 25 x 200 mm. test tube while the other plants were grown in 500 ml. Erlenmeyer flasks in a greenhouse maintained at 19°C. The addition of glucose to the nutrient medium did not appear to aid tuberization. Of the eight plants which produced tubers, three were grown on a medium containing 5 per cent glucose, one was grown on a medium containing 1 per cent glucose, and the nutrient of the remaining four contained no glucose. Leaf or stem tissue, roots and sand from each flask were plated out on yeast extract agar at the time of harvest to check for contamination. The fungus was present in the tubers produced by each of the eight plants. The remaining 368 plants that failed to produce tubers were not examined for the presence of the fungus.

Tubers were produced on plants originating from excised sprouts placed on a sterile substrate. Eleven tubers from eleven plants were examined and the fungus was present in each tuber.

Discussion

Fungi have been reported by various authors to form mycorrhizal associations with the roots of the potato plant (12). Bernard (2, 4), Constantin and Magrou (8) and Magrou (13, 15) have studied this association in detail. Non-specific endotrophic mycorrhizae were regularly associated with the roots of the potato plant and these authors believed that under normal conditions these fungi were associated with tuberization. The first report of a non-pathogenic fungus associated with potato tuber tissue was made by Cooper *et al* (7). The organism described by these authors, and which is the subject of this paper, is quite different in morphological and cultural characteristics from those previously described.

The organism under investigation appeared as a naked strand of protoplasm, and was best observed in freehand sections which were treated with a minimum of chemical agents. In such preparations it was possible to follow the strands through a considerable depth of host tissue. Stains commonly used to study fungi did not reveal hyphal walls. The absence of hyphal walls made this fungus difficult to detect, as there was no stained wall material to serve as an identifying character or to maintain the protoplasmic material in its original form. What appeared in individual microtomed sections was not readily recognizable as a fungus, due to the lack of stained hyphal walls and the probable action on the protoplasm of the chemical agents used in the paraffin and staining procedures.

The term fungus-like has been used in this presentation because of the lack of proof that what is being observed is in fact a fungus. Nuclei, cell walls and reproductive bodies were not observed. Neither was it

possible to isolate the fungus in pure culture independent of its host tissue. There was, however, considerable evidence that these structures were fungal hyphae. No structures of normal potato tissue which pass intercellularly and intracellularly through the tissue, and which appear as strands several hundred microns long have been previously described. Structures of this type were not present in the storage tissues of beet, carrot and sweet potato when examined under similar conditions.

The fact that fungus-free tissue cultures were established which appeared not to differ from fungous-infected cultures in other respects, indicated that these fungus-like structures were not a part of the potato tissue. Also cultures free of the fungus were established by incubating infected cultures on media containing antibiotics. In other cases infected cultures treated with antibiotics appeared to have reduced amounts of the fungus suggesting fungicidal interactions between a microorganism and materials known to possess antibiotic properties. This evidence indicated that the structures are living organisms intimately associated with, but separate from the host tissue. Although conclusive proof is lacking to establish the fact that the structures present in tuber and culture tissue are fungal hyphae, the evidence strongly indicates that this is the case.

The fungus persisted in cultures maintained through many monthly subcultures for a period of four years. This indicated that the fungus grew within the host tissue. If fungal growth did not take place, the amount of fungus originally introduced into the culture by the plant piece from which it was established would have been diluted by the fractionation of the cultures during transfers to a point where the fungus could no longer be readily observed.

Fungi were previously reported growing in association with tissue cultures of other species. Morel (19) reported the growth of *Plasmopara viticola* (Berk. and Curt.) Berl. and DeT. on cultured grape tissue. Gall tissue induced by *Gymnosporangium juniperi-virginianae* Schw. also grew in tissue culture (10). The vegetative mycelia of these fungi grew within and over the surface of the culture pieces.

The fungus in potato tissue cultures was not visible on the surface of the tissue culture piece. Hyphae were demonstrated microscopically in stained preparations to pass over the surface of the culture for a distance of 90 microns, and the fungus was most abundant in the non-meristematic regions near the surface of the culture.

Tissue cultures have been used to aid in the isolation and study of fungi, bacteria and viruses. Attempts to isolate this fungus from potato tissue cultures have failed. Modifications of the culture medium might induce the growth of the fungus in culture. However, the tissue culture technique has made it possible to indirectly examine the stems, rhizomes and leaf petioles of potato plants for the presence of the mycelium. In their natural form these plant parts contain too many chloroplasts for accurate inspection of the tissue.

Attempts were made to examine potato seed for the fungus using the freehand section technique. The hardness and small size of the seed prevented preparation of satisfactory seed sections. Evidence is available from three sources that indicates that this organism may be seed borne: 1, the fungus was present in tubers produced by plants grown from true

seed under aseptic conditions; 2, the fungus was observed in tissue cultures derived from plants grown from true seed under aseptic conditions; and 3, the fungus was present in tubers produced in culture from pieces of stem which had been removed from plants grown from true seed under aseptic conditions. Eleven such stem pieces were placed on the culture medium and tissue cultures were established. Three of these stem pieces produced rudimentary leaves, rhizomes and small tubers before active proliferation of callus tissue began.

In this study, and in the work reported by Cooper *et al*, a fungus mycelium was found present in every tuber examined. If this organism was not serving a purpose, if it were merely transient in the potato tissue, one would expect to find host tissue free of the fungus. Since this is not the case, it is possible that this organism may have entered into a symbiotic, mycorrhiza-like association with the host tissue. In the associations between fungi and potato roots reported by other authors, the potato was regarded as being dependent on the fungus while the fungus was thought to be relatively independent of the potato. It is evident that the organisms reported by previous workers to form associations with potato roots are quite different from the fungus reported here to be associated with the tubers. In this case the host tissue appears necessary to the fungus as attempts to separate the fungus from its host have failed; and it is possible, because of the ever-presence of the fungus in tuber tissue under normal conditions, that the fungus is beneficial to the host tissue.

Considerable progress has been made in obtaining differentiated plant tissues from callus cultures (17, 23). Fungus-free and fungus-infected tissue cultures may, therefore, prove valuable tools to clarify this host-fungus relationship.

Summary

1. A fungus-like structure was present in healthy tubers of *Solanum tuberosum* and 45 other *Solanum* species.

2. The fungus was present in all tubers examined.

3. A similar fungus was present in tissue cultures derived from the tubers, stems, rhizomes and leaf petioles of *Solanum tuberosum*.

4. Tissue cultures free of the fungus were produced.

5. The fungus failed to grow on media commonly used to isolate microorganisms.

6. The fungus was seed borne within surface sterilized potato seeds.

Acknowledgment

The author wishes to acknowledge his indebtedness to Dr. D. C. Cooper, Dr. A. C. Hildebrandt and Dr. G. H. Rieman for their advice and criticism during the course of this investigation. Appreciation is expressed to Eugene Herrling for preparing the figures.

Literature Cited

1. Barker, W. G. 1953. A method for the *in vitro* culturing of potato tubers. Science 118: 384-385.
2. Bernard, N. 1901. Sur la tuberculication de la Pomme de terre. Compt. rend. 132: 355-357.

3. ————. 1902. Études sur la tubérisation. Rev. gen. botan. 14: 5-25, *et seq.*
4. ————. 1911. Les mycorhizes des *Solanum*. Ann. sci. nat Botan. biol. végétale Seq. 9 14: 235-238.
5. ————, and J. Magrou. 1911. Sur les mycorhizes des Pommes de terre sauvages. Ann. sci. nat. Botan. biol. végétale Ser. 9 14: 252-258.
6. Castan, R. 1941. Recherches sur les conditions de tuberisation des stolons de Pomme de terre. Compt. rend. soc. biol. 135: 578-580.
7. Cooper, D. C., G. H. Rieman and R. W. Hougas. 1951. A mycorrhiza-like mycelium in potato tubers. 22nd Annual Report. The National Potato-Breeding Program, U.S.D.A.
8. Constantin, J. and J. Magrou. 1935. Sur les mycorhizes de la Pomme de terre. Ann. sci. nat. Botan. biol. végétale Ser. 10 17: 37-50.
9. Gerdemann, J. W. 1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infection. Mycologia 47: 619-632.
10. Hotson, H. H. and V. M. Cutler. 1951. The isolation and culture of *Gymnosporangium juniperi-virginianae* Schw. upon artificial media. Proc. Nat. Aca. Sci. U.S. 37: 400-403.
11. Jumelle, H. 1905. De l'influence des endophytes sur la tubérisation des *Solanum*. Rev. gen. botan. 17: 49-59.
12. Kelley, A. P. 1950. Mycotrophy in Plants. Chronica Botanica Co., Waltham, Mass.
13. Magrou, J. 1914. Symbiose et tubérisation chez la Pomme de terre. Compt. rend. 158: 50-53.
14. ————. 1938. Sur la tubérisation de la Pomme de terre. Compt. rend. soc. biol. 127: 793-796.
15. ————. 1944. Sur le rôle trophique des mycorhizes endotrophes. Compt. rend. 219: 519-521.
16. ————, J. Bouget and G. Segretain. 1943. Semis symbiotiques de Pomme de terre dans les Pyrénées. Compt. rend. 216: 501-503.
17. Miller, C. O., F. Skoog, M. H. Van Saltza and F. M. Strong. 1955. Kinetin, a cell division factor from deoxyribonucleic acid. J. Am. Chem. Soc. 77: 1392.
18. Molliard, M. 1915. Production expérimentale de tubercules aux dépens de la tige principale chez là Pomme de terre. Compt. rend. 161: 531-532.
19. Morel, G. 1944. Le developement du Mildiou sur des tissus de Vigne cultivés *in vitro*. Compt. rend. 218: 50-52.
20. Muir, W. H., A. C. Hildebrandt and A. J. Riker. 1954. Plant tissue cultures produced from single isolated cells. Science 119: 877-878.
21. Robbins, W. J. and Annette Hervey. 1951. Contaminating bacteria in plant tumor tissues. Science 114: 322-323.
22. Sanford, G. B. 1948. The occurrence of bacteria in normal potato plants and legumes. Scien. Agr. 28: 21-25.
23. Skoog, F. 1954. Substances involved in normal growth and differentiation of plants. Brookhaven Symposia in Biology 6: 1-21.
24. Smith, P. G. and J. C. Walker. 1941. Certain environal and nutritional factors affecting *Aphanomyces* root rot in garden pea. Jour. Agr. Res. 63: 1-20.
25. Steward, F. C. and S. M. Caplin. 1951. A tissue culture from potato tuber: The synergistic action of 2,4-D and of coconut milk. Science 113: 518-520.
26. Tervet, I. W. and J. P. Hollis. 1948. Bacteria in the storage organs of healthy plants. Phytopath. 38: 960-967.
27. Theis, T. N., A. J. Riker and O. N. Allen. 1950. The destruction of crown-gall bacteria in periwinkle by high temperature and high humidity. Amer. Jour. Bot. 37: 792-801.
28. Volcani, Zafrira, A. J. Riker and A. C. Hildebrandt. 1953. Destruction of various tissues in culture by certain bacteria. Phytopath. 43: 92-94.
29. Young, D. A., A. C. Hildebrandt, G. H. Rieman and D. C. Cooper. 1953. A mycorrhiza-like fungus in potato tissue cultures. (Abs.) Phytopath. 43: 490.

THE EFFECT OF GIBBERELIN COMPOUNDS ON THE SHAPE OF POTATO TUBERS[1]

D. J. MacLeod[2] and J. L. Howatt[3]

Interesting changes in the growth habit (1, 2), flowering and fruit setting (4) and dormancy (3) of plants have been attributed to the class of growth-promoting substances known as gibberelins. The following note deals with an additional effect produced by this class of compounds on developing potato tubers.

Fifteen Green Mountain plants grown under field conditions were chosen for this trial. The potatoes were planted May 22. The roots on one side only of each of 10 of these plants were partially bared on July 19 (when tuberization was just beginning) and stolon tips or terminal enlargements were brushed with potassium gibberelate (Gibrel, Merck) and gibberelic acid (Eli Lilly) respectively, each at a concentration of 20 p.p.m. At the same time the stolons of these plants were underlaid with paper toweling saturated with the two chemicals tested. The disturbed soil was immediately replaced. No evidence of wilting or any other abnormal reaction was observed following this treatment. In addition the vines were sprayed with approximately 0.5 and 1 liter of each of the chemicals on July 19 and 25, respectively. An examination of the plants on August 14 revealed that those treated with the chemicals were taller than the untreated checks and showed no evidence of bloom. The untreated plants had a profusion of flowers. The trials were harvested on October 2, and the results obtained are shown in the following photograph.

Referring to figure 1 it will be noted that all the potatoes produced on the plants treated with gibberelin compounds showed certain malformations, including elongated, spindled and dumbbell shaped tubers having numerous and shallow eyes with protruding brows. Noteworthy is the fact that most of these symptoms are characteristic of those generally associated with spindle tuber disease caused by a virus. According to the conditions of this test the yield of tubers was not affected adversely by the gibberelin compounds.

Literature Cited

1. Brian, P. W. and H. G. Hemming. 1955. The effect of gibberelic acid on shoot growth of pea seedlings. Physiol. Plantarum 8: 669-681.
2. Maramorosch, Karl. 1957. Reversal of virus caused stunting in plants by gibberellic acid. Science, Vol. 126, No. 3275: 651-652.
3. Rappaport, Lawrence. 1956. Growth regulating metabolites. Cal. Agr., Vol. 10, No. 12: 4-11.
4 Wittwer, S. H., M. J. Bukovac, H. M. Sell and L. E. Weller. 1957. Some effects of gibberelin on flowering and fruit setting. Plant Physiol. 32: 39-41.

[1]Accepted for publication January 15, 1958.
Contribution No. 1656 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2]Senior Plant Pathologist and
[3]Plant Pathologist, Plant Pathology Laboratory, Fredericton, New Brunswick, Canada.

FIGURE 1.—Effect of gibberelin on tuber shape: Left, treated with potassium gibberelate; Center, untreated check; Right, treated with gibberellic acid.

AMERICAN POTATO YEARBOOK

The 1958 edition of the AMERICAN POTATO YEARBOOK has just come off the press. The current volume contains 80 pages of vital information to the potato grower, shipper, jobber, agricultural teacher, research specialist and all others connected with potatoes in any way.

A special feature is the illustrated article on Potato Washing and Waxing by Herbert Findlen, U. S. Department of Agriculture, East Grand Forks, Minn. Also of significant interest are two pages of complete figures on potato acreage, yield, production, farm disposition and utilization in the U. S. from 1919 to 1956. There is in addition a current list of recent references to potato culture in the U. S. and Canada, comprehensive information on United States Standards for Potatoes and complete details on leading potato producing areas.

Other interesting items include rules and regulations affecting the shipment of seed potatoes, 1958 acreage guides, a list of leading United States and Canadian associations engaged in the improvement of potatoes, together with the names of United States and Canadian seed certification officials. The YEARBOOK also gives information on how and where to secure helpful brochures and leaflets covering all phases of the potato industry.

The new volume again contains a wealth of statistical information. There are tabulations of both seed and table stock production as well as statistics on Canadian and world production. There is also an article on "Selling, Merchandising and Distributing Potatoes," and a page devoted to "1957 Potato Highlights."

Copies of the YEARBOOK may be secured from the AMERICAN POTATO YEARBOOK, 8 Elm Street, Westfield, New Jersey. An individual copy sells for $2.00. A complete volume, 1950 - 1958, is available at $10.

POTASH AND BLACKSPOT

Preliminary results of controlled potash tests on potato crops in California's Kern County have been termed successful by University of California officials in charge of the program.

Effects of potash applications to prevent a type of physiological leaf roll and blackspot or stem end bruising in potatoes has been under study by the University for some time.

Dr. Oscar Lorenz, of the university, announced tentative results at a recent field day held near Bakersfield and attended by approximately 100 representatives of the chemical fertilizer industry, potato farmers and university officials.

Lorenz pointed out, however, the final evaluation would have to await harvesting and marketing of this year's potato crop.

Frank McGrane, agricultural chemicals sales manager for American Potash & Chemical Corporation, said the company was "highly encouraged" by the results reported to date.

"One of the subjects under study is potash ability to prevent spoilage during transit to eastern markets," McGrane said. "Inasmuch as this may run as high as 30 per cent, it is extremely important that some way be found to cut it down.

The problems of potato stem end blackening and physiological leaf roll are relatively new in this area. However, stem end blackening has been known for many years in Holland where heavy potash applications have corrected the problem. In some areas of the United States, potash has reduced but not eliminated the condition. Blackening occurs only in tissues of the stem end of tubers which have received bruising. It may occur only at the periphery of the tuber or may extend into the tuber for an inch or more.

While the blackspot takes its toll after the crop is harvested, leaf roll goes hand-in-hand with lower crop yields. It is not yet known what the exact cause or nature of leaf roll is, but it is known that potato output drops considerably when this type of leaf roll exists.

McGrane said a number of railroad cars of Kern County potatoes were rejected at eastern cities last season because of blackspot. He said tests to date indicate potash will cut down on this loss, as well as a type of physiological leaf roll.

The current testing program in Kern County follows two-year tests made in the Santa Maria and Madera (Calif.) areas in 1955 and 1956 to correct potash deficiency in crops. Blackspot in potatoes was a problem at Santa Maria, while general potash deficiency existed in both regions.

Since the successful conclusion of these tests potash consumption has increased greatly in both areas, with a subsequent correcting of the condition, according to McGrane.

THE 42ND ANNUAL MEETING
THE POTATO ASSOCIATION OF AMERICA
in conjunction with A.I.B.S.
INDIANA UNIVERSITY, BLOOMINGTON
August 25 - 27, 1958
——Information on Housing and Meals——

RESIDENCE HALLS ACCOMMODATIONS will be available for single persons, couples, and families. Those staying three or four nights will be assigned on the American Plan. Those staying not more than two nights will be assigned on the European Plan, which will also be available for persons participating in the pre- and post-meeting activities.

THE AMERICAN PLAN is a "package" plan of room and meals in an assigned dormitory. Rates are 6.00 per day, $18.00 minimum, for adults; $4.75 per day, $14.25 minimum, for children 7 through 17; $2.85 per day, $8.55 minimum, for children under 7. The American Plan covers the period from dinner on August 24 through luncheon on August 28. Rooms must be vacated by afternoon of August 28. Persons on this plan may occupy their rooms on the night of August 23 at the American Plan rate of $2.25. Persons staying for the night of August 28 or 29 will be moved to the European Plan housing center for these nights.

THE EUROPEAN PLAN is for rooms without meals and will be available for pre- and post-meeting activities and for a maximum of two nights during the regular meetings. Rates are $2.50 per person per night in a room with twin beds, $3.00 for a single room. Meals will be available in the Memorial Union as well as in local restaurants and hotels. Facilities are available for the storage of luggage without cost during the pre- and post-meeting periods.

OTHER HOUSING ACCOMMODATIONS will be available on the campus in the Campus Club (part of the Memorial Union), local hotels and motels, as well as other motels and the hotel and cabins in two state parks within a radius of twenty miles. Rates in the Campus Club are $4.50 for a single room, $5.50 with double bed, $7.50 with twin beds. All rooms are equipped with lavatories and are air-conditioned. Rates in the local hotels and motels compare favorably with those of similar quality in the Middle West generally. Campsites are available in the two state parks which are within twenty miles of the University.

RESERVATIONS for all housing, except campsites in state parks, should be returned as soon as possible on the attached form. **Do not send money with the pre-registration.** Those wishing to share a room with a particular person or to be assigned with a particular group should so indicate on the reservation form.

BANQUETS. The full price of banquets served on the campus will not exceed $3.00. Persons on the American Plan will be given a credit of 50 cents for breakfast or luncheon and 75 cents for on-campus banquets at the time the banquet tickets are purchased.

ACKNOWLEDGMENT of each advance registration **received before August 9** will be made on a form which should be presented on arrival when registration is completed.

PRE-REGISTRATION IS ESSENTIAL TO PROPER ASSIGNMENT OF FACILITIES

REGISTRATION AND HOUSING APPLICATION

Name ..... ☐ Male ☐ Female
(First) (Middle) (Surname) ☐ Graduate Student

Home .....
(Street) (City) (State)

Full names, sex, relationship of members, ages of children in party:

.....

.....

.....

Professional Address .....

Title .....

Institution .....

Society Membership:
(1) .....
(2) .....
(3) .....

I plan to attend the following society banquets .....

.....

Expected time of arrival at Indiana University:

.....
(Date and Hour)

☐ By train at Bloomington
☐ By plane at Bloomington
☐ By plane at Indianapolis (Limousine service available to Bloomington)
☐ By bus at Bloomington
☐ By car

Departure from Bloomington:

.....
(Date & Hour)

☐ Please make reservations for me in a University Resident Hall

I prefer housing in a
☐ Hotel ☐ Campus Club
☐ Motel
☐ I will not require housing

Remarks: .....

.....

.....

I plan to take the following field trips .....
(Applies to Graduate Students only)

Signature of major professor .....

Please print or type the above information and mail to:

EDUCATIONAL CONFERENCE BUREAU
Indiana Memorial Union
Bloomington, Indiana

Date.....

Harvest more No. 1 potatoes with these versatile Du Pont fungicides

PARZATE®—

liquid nabam fungicide

the sure, simple way to control both blights of potatoes

You get higher yields of Number 1 potatoes at lower spray cost with Du Pont "Parzate." Easy to use, "Parzate" simplifies your disease control by stopping both early and late blights — even under the worst conditions. "Parzate" resists washing off by rains, and can be used under all weather conditions. And "Parzate" won't stunt plants. It's tough on diseases, but mild on plants — allows maximum growth and yields. Its long record of success makes "Parzate" your most effective, reliable way to control potato diseases.

MANZATE®—

maneb fungicide

the newest fungicide from Du Pont to control diseases of potatoes

Grower trials and research have shown that "Manzate" gives highly effective control of early and late blight of potatoes. "Manzate" is a wettable powder that readily mixes with water for uniform sprays. It is compatible with commonly used insecticides and mild on plants. This year, on your potatoes, see the high degree of disease control you get with "Manzate"; try it on one field all season.

DU PONT

REG. U.S. PAT. OFF.

on all chemicals, always follow label instructions and warnings carefully

BETTER THINGS FOR BETTER LIVING . . . THROUGH CHEMISTRY